

FINAL REPORT

Characterization of the Fate and Biotransformation of
Fluorochemicals in AFFF-Contaminated Groundwater at
Fire/Crash Testing Military Sites

SERDP Project ER-2128

APRIL 2017

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REPORT DOCUMENTATION PAGE

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1. REPORT DATE (DD-MM-YYYY) 04-10-2017		2. REPORT TYPE Final Report		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE Characterization of the Fate and Biotransformation of Fluorochemicals in AFFF-Contaminated Groundwater at Fire/Crash Testing Military Sites				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jennifer Field				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Oregon State University 1007 Agricultural and Life Sciences Building Corvallis, OR 97331				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Strategic Environmental Research and Development Program 4800 Mark Center Drive, Suite 17D03 Alexandria, VA 22350				10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) ER-2128	
12. DISTRIBUTION / AVAILABILITY STATEMENT Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The overall objective of this study is to fully delineate the per- and polyfluoroalkyl substances (PFASs) that persist in aqueous film-forming foam (AFFF)-contaminated groundwater, sediment, and soil and evaluate their impact on priority pollutant biotransformation. We intend to characterize the composition of individual PFASs and their precursors in AFFF formulations and delineate the total organic fluorine concentrations of AFFF-contaminated groundwater, sediment, and soils. To achieve this overall goal, we had the following technical objectives (Tasks): 1. Characterize the concentration/composition of individual PFASs and their precursors in AFFF formulations. 2. Characterize the individual PFASs and total organic fluorine composition of AFFF-contaminated groundwater, sediment, and soil at military fire-training sites; determine field-based estimates of PFAS transport; and evaluate the spatial relations with priority pollutant distributions in groundwater at military fire-training sites. 3. Determine the potential for the biotransformation of partially-fluorinated substances and AFFF formulations and the impact of AFFF and its components on TCE transformation under redox conditions that are representative of groundwater at fire-training sites. 4. Characterize the					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:		17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Jennifer Field	
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	U	63	19b. TELEPHONE NUMBER (include area code) 541-737-2265

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ABBREVIATIONS AND ACRONYMS

AEC	Anion exchange capacity
AFB	Air Force Base
FFFF	Aqueous film forming foam
CEC	Cation exchange capacity
CO ₂	carbon dioxide
DGBE	Diethylene glycol butyl ether
ECF	Electrofluorination
FAB-MS	Fast atom bombardment mass spectrometry
FOSA	Perfluorooctane sulfonamide
FtB	Fluorotelomer betaines
FTCA	Fluorotelomer carboxylates
FT-ICR	Fourier transform ion cyclotron resonance
FTSaB	Fluorotelomer sulfonamido betaine
FTAoS	Fluorotelomer thioamido sulfonate
FTSAOAOs	Fluorotelomer sulfoxide amido sulfonate
FTSAO ₂ AoS	Fluorotelomer sulfone amido sulfonate
FTTHN	Fluorotelomer thio hydroxy ammonium
FTTPA	Fluorotelomer thioether propanyl alaninate
FTSaAm	Fluorotelomer sulfonamide amine
FTSA	Fluorotelomer sulfonate
FTUCA	Fluorotelomer unsaturated carboxylate
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
K _d	Soil:water partition coefficient
K _f	Freundlich sorption coefficient
K _{oc}	Organic carbon:water partition coefficient
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MilSpec	Military specification
N ₂	Nitrogen
NaOH	sodium hydroxide
NH ₄ OH	Ammonium hydroxide
pKa	Acid dissociation constant
PFASs	Perfluoroalkyl substances
PFBA	Perfluorobutanoate
PFBS	Perfluorobutane sulfonate
PFCA	Perfluorinated carboxylate
PFDA	Perfluorodecanoate
PFDoA	Perfluorododecanoate
PFDS	Perfluorodecane sulfonate
PFEtS	Perfluoroethane sulfonate
PFHpA	Perfluoroheptanoate
PFHpS	Perfluoroheptane sulfonate
PFHxA	Perfluorohexanoate
PFHxS	Perfluorohexane sulfonate
PFHxSA	Perfluorohexane sulfonamide

PFNA	Perfluororononanoate
PFNS	Perfluororonane sulfonate
PFOA	Perfluorooctanoate
PFOS	Perfluorooctane sulfonate
PFPeA	Perfluoropentanoate
PFPeS	Perfluoropentane sulfonate
PFPrS	Perfluoropropane sulfonate
PFSA	Perfluoroalkyl sulfonate
PFSaAm	Perfluoroalkyl sulfonamido amine
PFSaAmA	Perfluoroalkyl sulfonamide amino carboxylate
PFTrA	Perfluorotridecanoate
PFTeA	Perfluorotetradecanoate
PFUnA	Perfluoroundecanoate
QPL	Qualified products list
QTOF	Quadrupole time of flight
rDNA	Ribosomal deoxyribonucleic acid
TCE	Trichloroethene
TOP	Total oxidizable precursor

ACKNOWLEDGMENTS

The research described in this final report was supported by the US Department of Defense, through the strategic environmental research and development program (SERDP) Dr. Andrea Leeson and other SERDP staff are knowledge for their assistance and support.

EXECUTIVE SUMMARY

Objectives: The overall objective of this study is to fully delineate the per- and polyfluoroalkyl substances (PFASs) that persist in aqueous film-forming foam (AFFF)-contaminated groundwater, sediment, and soil and evaluate their impact on priority pollutant biotransformation. We intend to characterize the composition of individual PFASs and their precursors in AFFF formulations and delineate the total organic fluorine concentrations of AFFF-contaminated groundwater, sediment, and soils. To achieve this overall goal, we had the following technical objectives (Tasks):

1. Characterize the concentration/composition of individual PFASs and their precursors in AFFF formulations.
2. Characterize the individual PFASs and total organic fluorine composition of AFFF-contaminated groundwater, sediment, and soil at military fire-training sites; determine field-based estimates of PFAS transport; and evaluate the spatial relations with priority pollutant distributions in groundwater at military fire-training sites.
3. Determine the potential for the biotransformation of partially-fluorinated substances and AFFF formulations and the impact of AFFF and its components on TCE transformation under redox conditions that are representative of groundwater at fire-training sites.
4. Characterize the sorption of cationic and zwitterionic PFASs to soils and sediments.

Technical Approach. For **Task 1**, the PFAS composition of 3M and fluorotelomer-based AFFFs was determined using a number of mass spectrometric approaches. The total oxidizable precursor assay was adapted for use with AFFF-contaminated groundwater, soil, and sediment. For **Task 2**, the developed analytical tools were applied to environmental samples from US military bases. Microcosm experiments were performed for **Task 3** to determine the biotransformation pathways of polyfluoroalkyl substances in fluorotelomer-based Ansul AFFF under anaerobic and aerobic conditions. The impact on TCE reductive dechlorination was also evaluated in laboratory batch microcosm experiments. For **Task 4**, a series of laboratory batch sorption experiments were conducted using a fluorotelomer-based AFFF from National Foam, which contained anionic, zwitterionic, and cationic PFASs, on six soils that varied in physical properties.

Results. Over 50 classes of PFASs, comprised of several individual homologs, were identified in AFFF formulations and groundwater over the course of this project. The composition of AFFFs was defined as either 3M or fluorotelomer-based AFFFs (Ansul, Chemguard, National Foam, Buckeye Fire Equipment, and Angus) with dates of manufacture dating back to 1989. Many of the newly-identified classes are 3M-derived and are cationic or zwitterionic. The TOP assay was modified for use with AFFF-contaminated media and precursors make up a significant fraction of total PFASs in groundwater, soil, and sediment. However, perfluoroalkyl carboxylates (PFCAs) (e.g., perfluooctanoate, PFOA), perfluoroalkyl sulfonates (PFSAs) (e.g., perfluorooctane sulfonate, PFOS), and fluorotelomer sulfonates (FTSAs) remain the most abundant individual PFASs in AFFF-contaminated groundwater. The biotransformation of fluorotelomer thioamido sulfonates (FtTAoS) occurs under anaerobic and aerobic conditions; however, the two conditions produce different transformation products. Aerobic conditions produced FTSAs; however, they were further transformed to PFCAs. Some AFFFs and the solvent present in AFFF, diethyl glycol butyl ether (DGBE) promoted reductive dechlorination of trichloroethene when provided as the sole carbon and energy source. Low K_d values for the anionic 6:2 FTSA in sorption experiments are consistent with the detection and mobility of 6:2

SA is groundwater. Higher K_d values indicate that the anionic 8:2 FTSA, zwitterionic FTSaBs, and cationic 6:2 fluorotelomer sulfonamido amine (FTSaAm) are more likely to be associated with soil and sediment of source zones. Complete removal of the cationic FTSaAm indicates potential for strong sorption to source zone soils and sediments at some sites. The lack of correlations between the sorption of anionic FTSaS, zwitterionic FTSaBs, and cationic 6:2 FTSaAm and parameters including organic carbon content, CEC, and AEC, indicates that the bulk parameters do not adequately predict sorption. More information is needed on the conditions (e.g. pH and ionic strength) that promote desorption of zwitterionic and cationic PFASs in order to determine the potential for source zone soils and sediments to act as long-term PFAS sources.

Benefits: The analytical tools developed for this project, including methods for quantifying individual PFASs as well as precursors by the total oxidizable precursor (TOP) assay, we have provided analytical advances for more complete characterization of AFFF-contaminated media. Using these tools, we have generated information that has significantly improved our understanding of the PFASs present in groundwater, sediment, and soil at AFFF-contaminated sites. Having identified precursors at AFFF-contaminated sites, efforts now focus on understanding the process that retain PFASs in source zones and the conditions that may mobilize them. Identifying precursors will lead to a better of understanding of the effectiveness of treatment technologies, such as the use of granulated activated carbon and other sorbents for their removal. The biotransformation pathway of the polyfluoroalkyl substances in Ansul AFFF provides a framework for understanding the fate of the precursor and insight into the conditions (anaerobic) that lead to high concentrations of persistent FTSaS and the potential for intermediates to be ultimately transformed to persistent PFCAs.

Transition Plan: We worked closely with Wellington Laboratories to identify and name new PFASs and to advocate for the synthesis of high quality authentic standards for perfluoroethane sulfonate (PFEtS). We participated in a number of webinars with DoD participants. Information gained from this project was used to inform current SERDP and ESTCP projects including SERD ER- ER-2720 and ESTCP 201633.

1 PROJECT OBJECTIVE AND TASKS

2 The overall goal of this project is to better understand the occurrence, behavior, and transport of
3 per- and polyfluoroalkyl substances (PFASs) that are associated with aqueous film forming foam
4 (AFFF) and AFFF-contaminated groundwater, sediment, and soil at military sites where fire-
5 training activities or crashes have occurred and to evaluate their impact on trichloroethene (TCE)
6 biotransformation.

7 To achieve this overall goal, the four specific tasks of this SERDP project are to:

- 8 1. characterize the concentration/composition of individual PFASs and their precursors in AFFF
9 formulations,
- 10 2. characterize the individual PFASs and total organic fluorine composition of AFFF-
11 contaminated groundwater, sediment, and soil at military fire-training sites and to evaluate
12 the spatial relations of PFASs with priority pollutants at military fire-training sites, and
- 13 3. determine the biotransformation of partially-fluorinated substances and other AFFF
14 formulation components and the relation with TCE transformation under redox conditions
15 relevant to fire-training/crash sites.
- 16 4. characterize the sorption of cationic and zwitterionic PFASs

17 This final report for SERDP ER-2128 describes the activities conducted to complete **Task 1**
18 (characterization of AFFF), **Task 2** (groundwater, sediment/soil characterization and spatial
19 relations of PFASs and priority pollutants at a field site), **Task 3** (biodegradation of AFFF
20 components in relation to TCE biodegradation), and **Task 4** (sorption of cationic and
21 zwitterionic PFASs). Completion of these tasks addresses the Statement of Need (ERSON—11-
22 02) because the information provided will improve our fundamental understanding of the
23 identity, fate, and transport of PFASs at AFFF-contaminated at military sites and their impact on
24 the biotransformation of the chlorinated solvent, TCE.

25 BACKGROUND

26 **Task 1.** From the early 1960s until present, Military Specification (MilSpec) requirements and
27 AFFF formulations sold on MilSpec have changed. The original MilSpec specified only
28 performance parameters (e.g., surface tension) but ‘environmental limits’ were added in 1977
29 with upper limits set < 500,000 mg/L for biological and chemical oxygen demand. By 1981,
30 toxicity limits were in place.⁷ Two main types of AFFFs (6% and 3%) are qualified under US
31 MilSpec. The composition of the 3 and 6% types are similar for a given manufacturer, with the
32 difference being the degree of dilution required to achieve the performance speculations. For
33 example, 6% AFFF product must be diluted to 6% with water and 3% AFFF formulations must
34 be diluted to 3% with water. The U.S. Navy developed and uses the equipment necessary to
35 deliver 6% AFFF while the US Air Force primarily uses equipment aimed at delivering 3%
36 AFFF. MilSpec history indicates that AFFFs were sold by 3M from the mid 1960 to the early
37 1970s and were the sole source of AFFF to the US military.⁶ In 1973, National Foam introduced
38 a MilSpec AFFF and in 1976 Ansul qualified an AFFF for MilSpec while it wasn’t until 1994
39 that other fluorotelomer-based AFFFs were introduced by Angus, Chemguard, Buckeye, and Fire
40 Service Plus.^{8,9} In 2015 and 2016, AFFFs were placed on the QPL by ICL, Tyco/Ansul,

41 Amerex/Solberg.^{8,9}

42 Once water is added, the 3 and 6% AFFF formulations for a given manufacturer give similar
43 PFAS concentrations in the final diluted product. From 1970 to present, multiple manufacturers
44 released multiple AFFFs that met MilSpec and that were placed on the qualified products list
45 (QPL).^{8,9} At the onset of this SERDP project it was not known if field sites would be
46 contaminated by a wide array of PFASs or only a few. Therefore, Task 1 was designed to
47 reverse engineer the proprietary PFAS composition of AFFF formulations stockpiled and
48 actually used at field sites using multiple complementary analytical approaches.

49 **Task 2.** After use in firefighting and in training exercises, AFFF waste frequently was
50 discharged to surface-holding ponds or groundwater along with residual solvents that were not
51 consumed during combustion. On the basis of a limited amount of monitoring data, it appears
52 that perfluorooctanoate (PFOA), perfluorooctane sulfonates (PFOS), and Fluorotelomer
53 sulfonates (FTSAs) are transported in groundwater from the initial locations of contamination.
54 For example, our previous monitoring efforts¹⁰ at Tyndall AFB, Wurtsmith AFB, and NAS
55 Fallon indicate very high concentrations (e.g., 14.6 mg/L) within 50-120 m of fire-training pads,
56 with transport up to 540 m with concentrations of 4-9 µg/L that remain above health advisory
57 levels of 1 µg/L.¹¹ These areas also may serve as reservoirs for fluorinated compounds because
58 partially-fluorinated precursors of the more persistent forms have the potential to adsorb onto
59 soils and aquifer materials. For example, amphoteric surfactants have cationic functionalities
60 that may undergo cation exchange reactions onto sediments and soils. Furthermore, the high
61 biological oxygen demand loads entering the subsurface are likely to have resulted in strongly
62 reducing conditions in the areas of highest contamination. Under such conditions it is likely that
63 many of the biological processes that could result in release of the persistent perfluoroalkyl
64 carboxylates (PFCAs) (e.g., PFOA), perfluoroalkyl sulfonates (PFSAs) (e.g., PFOS), and FTSA
65 forms will slow down. Thus, any effort to remediate AFFF-contaminated groundwater would
66 need to address contaminated source zones even if they do not contain elevated concentrations of
67 PFOA, PFOS, and FTSA. To assess the potential for source zones to serve as reservoirs of
68 precursors, Task 2 was designed to measure concentrations of PFASs and the precursors in
69 groundwater and soils and sediments collected from areas immediately adjacent to locations
70 where runoff from fire-fighting activities was discharged to the subsurface.

71 **Task 3.** Previous research has established the fact that PFCAs and PFSAs are stable with respect
72 to biotransformation. Available data also suggest that PFSAs do not undergo biotransformation
73 at appreciable rates, but the number of prior studies that investigated this issue is limited, and no
74 prior studies address the anaerobic conditions typically encountered in AFFF-contaminated
75 groundwater. FTSAs and related compounds (e.g., fluorotelomer thioamidosulfonates or
76 FTTAoS) contain functional groups that are amenable to biotransformation. The FTSAs
77 undergo aerobic biotransformation to form PFCAs.¹² Only recently were FTSAs to be persistent
78 under anaerobic conditions.¹³ Prior to the onset of this project, no information was available on
79 the biotransformation of FTTAoS but formation of FTSAs from FTTAoS has been proposed.¹⁰
80 Therefore, Ansul AFFFs that contained FTTAoS and 3M AFFF were identified for study in this
81 project.

82 The rates of transformation and the identity of the microbial communities responsible for these
83 reactions and product yields have never been investigated. To date, a few studies report the

84 biologically-mediated production of PFOS from partially-fluorinated sulfonamide-based
85 substances.¹⁴ However, potential abiotic processes were not investigated and the associated
86 microbial community was not characterized. Therefore, because it is critical to understand the
87 mechanisms of AFFF transformation in the subsurface under a variety of relevant environmental
88 conditions and because it may be advantageous to stimulate key reactions prior to application of
89 other remedial approaches (e.g., activated carbon), we will evaluate the biodegradability of
90 PFASs within AFFF and associated solvents under redox conditions representative of
91 groundwater at fire/crash training sites. Task 3 was to determine the biodegradability of
92 polyfluoroalkyl substances in the AFFF formulation produced by Ansul under anaerobic and
93 aerobic conditions and in the presence of TCE.

94 The application of AFFFs to extinguish chlorinated solvent-based fires resulted in co-
95 contamination of PFASs and chlorinated solvents, such as TCE, in groundwater and soil.
96 Although reductive dechlorination of TCE by *Dehalococcoides mccartyi* is a frequently-used
97 remediation strategy, the effects of AFFF and PFASs on TCE dechlorination are not well
98 understood. AFFFs contain PFASs and non-fluorinated hydrocarbon surfactants and one or
99 more glycol ether-based solvents (8-20%) that were used to quickly verify that the target mixing
100 ratios were achieved prior to the application of AFFF to fires.¹⁵ Historically, the most commonly
101 used solvent was diethylene glycol butyl ether (DGBE). Because chlorinated solvents were
102 frequently used to create fires for fire-fighter training exercises, AFFF waste water must have
103 been comprised of PFASs and unburned solvents, such as TCE.^{15,16} Thus, many fire-fighter
104 training areas are likely co-contaminated by TCE and PFASs.
105

106 In situ remediation strategies for TCE-contaminated groundwater is conducted by promoting the
107 reductive dechlorination of TCE¹⁷ by adding substrates that ferment to generate hydrogen and
108 acetate.¹⁸⁻²¹ Repeated AFFF applications at training sites resulted in the repeat addition of AFFF
109 containing fermentable substrates, such as the DGBE and, in doing so, created reducing
110 conditions in subsurface environments that support TCE reductive dechlorination by *D.*
111 *mccartyi*. Prior to the onset of this project, the impacts of AFFF on dechlorination by *D.*
112 *mccartyi* had not been described. To address this data gap in Task 3, we investigated the impacts
113 of three AFFF formulations and of DGBE by itself on the reductive dechlorination of TCE.
114

115 **Task 4.** The sorption of PFCAs and PFSAs increases with the organic carbon content in soils
116 and sediments,^{22,23} as measured by solid-water partition coefficients (K_d) and organic carbon
117 normalized solid-water partition coefficients (K_{oc}). When organic carbon is low (0-0.78%),
118 PFSA and PFCA sorption is due to electrostatic interactions with mineral phases.^{24,25} Within the
119 PFCA or PFSA homologous series, longer-chained homologs have higher partition coefficients
120 than short-chained homologs.^{22,23,26,27}

121 In contrast, the anionic FTSAs and zwitterionic and cationic PFASs present in groundwater,²
122 soil,²⁸ and sediments²⁹⁻³¹ have received little attention. Two studies report field-derived K_d
123 values for the 6:2 FTSAs,^{29,32} while only one study reports field-derived K_d values for the
124 zwitterionic 6:2 fluorotelomer sulfonamido betaine (FTSaB) and the cationic 6:2 fluorotelomer
125 sulfonamido amine (FTSaAm).²⁹

126 In laboratory batch sorption experiments with PFCAs and PFSAs, multi-compound studies are
127 executed under controlled conditions.^{22,23,33} However, a similar experimental set-up for sorption

128 of zwitterionic and cationic PFASs is challenging due to the lack of authentic standards and
129 mass-labeled internal standards for the zwitterionic and cationic PFASs, and few reference
130 materials are available.² Furthermore, mass balance of the PFCAs and PFSAs is typically
131 achieved by extracting the soil or sediment.^{22,23} To date, all studies examining concentrations of
132 zwitterionic and cationic PFASs on soil and sediment have relied on a similar extraction method,
133 which has only been validated with methanolic standards added to dry soil¹ However, previous
134 studies of organic cations in soil and sediment have suggested that stronger extraction conditions
135 are typically necessary, particularly if the cations have been allowed to equilibrate with the soil
136 and/or sediment.³⁴ If a stronger soil extraction holds true for zwitterionic and cationic PFASs,
137 then both concentrations^{1,30,31} and field-derived K_d estimates²⁹ of zwitterionic and cationic
138 PFASs may be significantly underestimated due to incomplete extraction of the (presumably)
139 equilibrated soil and sediment.

140 Due to the complexity of AFFFs and AFFF-impacted field sites, one study attempted to simulate
141 the conditions of an AFFF-impacted fire training area by conducting sorption experiments with a
142 mixture of PFCAs and PFSAs with and without a non-aqueous phase liquid.²² Several studies
143 have examined the impact of PFCA and PFSA sorption on the presence of ionized hydrocarbon
144 surfactants,^{22,35} which only partially represents an AFFF discharge event. One study found an
145 increase in the sorption of a single compound, PFOS, in the presence of a single cationic
146 surfactant, while a single anionic surfactant decreased sorption of PFOS (single compound).³⁵
147 However, a single sorbate was probed by a single surfactant, which may have a greater sorption
148 impact than the presence of many sorbates (i.e. an AFFF). By contrast, a different anionic
149 surfactant caused no change or a slight decrease in sorption for long-chained ($n \geq 6$)
150 perfluoroalkyl acids when present in a mixture of PFASs.²² However, the scenario doesn't fully
151 capture the complexity of AFFFs, which contain various solvents and a suite of hydrocarbon
152 surfactants in addition to a mixture of PFASs.¹⁵

153 To best replicate the first application of AFFF to pristine soils at a field site, National Foam
154 AFFF, which contains anionic FTSAs, zwitterionic FTSaBs, and cationic FTSaAms (Fig. 2),^{2,6}
155 was selected for use in batch sorption experiments. National Foam AFFF was chosen since it
156 was approved for use by the U.S. military since 1976⁶ and contains a diverse array of anionic,
157 zwitterionic, and cationic PFASs. To determine the soil properties that drive sorption of the
158 anionic FTSAs, the zwitterionic FTSaBs, and the cationic 6:2 FTSaAm, blank soils were selected
159 to encompass a range of organic carbon, cation exchange capacity (CEC), and anion exchange
160 capacity while constraining soil pH to ~ pH 5. Since the FTSaBs and the 6:2 FTSaAm have
161 ionizable functional groups that change speciation with pH (Fig. 2), ion exchange may drive
162 sorption in a manner analogous to that of zwitterionic pharmaceuticals.^{36,37} given these data
163 gaps, Task 4 was to characterize the sorption behavior of cationic and zwitterionic on
164 soils/sediments and Task 5 was to model the sorption characterized in Task 4.

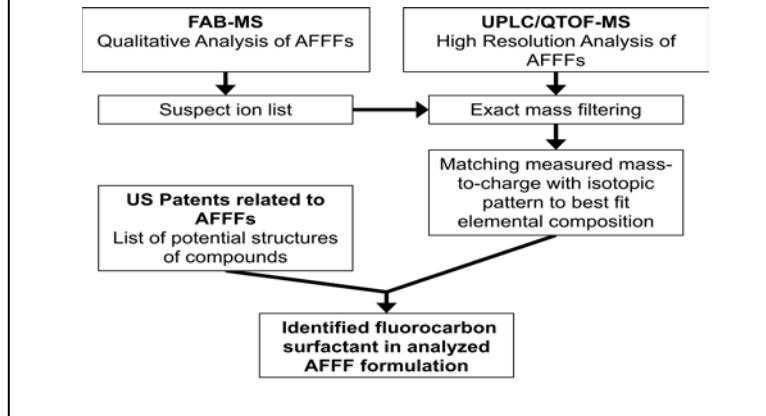
MATERIALS AND METHODS

Task 1: Characterize AFFF Formulations

Donald Warner, the Fire Chief of the Air Force, assisted our SERDP project by helping to organize the collection of AFFF samples from Air Force bases around the United States. With his assistance, we received shipment of 65 individual samples of AFFF that are currently stored at Air Force and Naval Bases in the United States. We now have a complete archive of AFFF formulations that are listed on the QPL that meet current Mil Spec. There are six manufacturers of AFFF that have products on the QPL including 3M (although their AFFF formulations are no longer manufactured), Angus, Ansul, Buckeye, Chemguard, and Kidde Fire Fighting. In addition, we have learned that several bases received AFFF formulations that were not listed on the Mil Spec, including First Strike and FireAid.

The strategy for **Task 1.1** was first to define the composition of AFFF formulations by fast atom bombardment mass spectrometry (FAB/MS) and LC with high mass accuracy quadrupole time of flight (QTOF) MS (Fig 1). FAB/MS was used to characterize the major PFASs and their respective fluorinated chain-length distributions in AFFF formulations. FAB/MS is rapid, inexpensive, and ideally suited for the detection of surfactant classes.^{38,39} LC-QTOF analyses were performed to determine the identity of unknown classes of PFASs in AFFF formulations. The identification of the newly-identified PFASs was then checked against patent information on AFFFs.⁶ LC-MS/MS, the most commonly applied analytical technique for the determination of PFASs, was used to identify the minor classes of PFASs in the AFFF formulations.

Fig 1. Scheme to identify new fluorochemicals in AFFF formulations.⁶



After the 2010 publication by Place et al.⁶, additional research was undertaken to identify unknown PFASs in AFFFs and groundwater. High-mass accuracy QTOF mass spectrometry was applied to samples of AFFF and groundwater.^{40,41} An ABS Science Triple TOF 5600 instrument, fitted with electrospray ionization was operated in negative ion mode, was used for measurements was located at the Colorado School of Mines, under the direction of Dr. Christopher Higgins (PI, SERDP ER-2126).

For **Task 1.1**, an extraction method was developed for the analysis of groundwater for the array of newly-identified PFASs.² Preliminary work indicated that large volume, direct injection does not provide benefits for PFAS analysis over solid phase extraction.⁴² However, we found that direct aqueous injection was not appropriate for water samples containing C8 and longer-chained

PFASs because the longer-chained forms partition to the air-water interface or to autosampler vials while samples are waiting for analysis. Because PFASs identified in Task 1.1 ranged in chain length from two carbons up to 14 carbons, new extraction and separation approaches were needed, since hydrophobic interactions with C-18-based solid phase extraction media and analytical columns is not sufficient to isolate and separate short-chained PFASs. We found that by adding salt and acidifying a small (3 mL) volume of groundwater, a small volume of organic solvent (ethyl acetate and trifluoroethanol) efficiently extracted anionic, zwitterionic, and cationic PFASs. In addition, an ion-exchange separation system was developed to concentrate short-chained PFASs with various charged (anionic, zwitterionic, and cationic) head groups. An orthogonal chromatographic system was developed for the separation and quantification of 49 individual PFASs found in AFFFs.^{2,6} The extraction and chromatographic methods were optimized, validated, and applied for analysis of groundwater samples for task 2.1.²

For **Task 1.2**, the total oxidizable precursor (TOP) assay was developed and then applied to characterize the production of dead-end products of the partially-fluorinated precursors present in the AFFF formulations. The US military AFFF formulations were analyzed by the TOP assay to determine precursor concentrations by first diluting the AFFF formulations 10,000 fold and then oxidizing them with potassium persulfate under highly basic conditions in the presence of heat. The hydroxyl radicals produced during the oxidation step are unselective oxidants that are capable of oxidizing almost every type of organic compound. PFCAs and PFSAs are among the limited number of compounds that are essentially unreactive with hydroxyl radical^{43,44}. Thus, measurements of the increase in concentrations of PFOS, PFOA, and related compounds after the oxidation step should provide a measure of the concentrations of precursors.

Because the zwitterionic and cationic PFASs were discovered during this project, extraction methods for sediment/soil needed to be developed for these PFASs. Existing methods for soils and sediments⁴⁵ were adapted to include the newly-discovered PFASs. A number of individual standards were added to analytical methods and the conversion of individual standards to PFCAs under TOP assay conditions was assessed.¹

Task 2: Characterize Groundwater, Soil, and Sediment at AFFF-Contaminated Sites

For **Task 2.1**, a limited number of groundwater samples collected from Ellsworth and Randolph Air Force Bases (AFB) were analyzed by the quantitative analytical method developed for Task 1.1.

For **Task 2.2**, Groundwater (n=26) was collected in 2011 from a 1200 m by 600 m area encompassing the burn pit. The groundwater depth was 2 to 8 m below ground surface. Groundwater was stored at 4°C prior to analysis. Twenty-two groundwater samples were analyzed. A 500-µL aliquot of groundwater collected from 5-cm below the liquid surface was added to 500 µL of methanol in a 2-mL microcentrifuge tube. Samples were centrifuged at 15,000 rpm for 5 min. Stable-isotope labeled internal standards were added to groundwater after the final dilution.

Soil (n=16) samples were collected 0.6 m below ground surface and sediments (n=10) were collected approximately 5 to 6 m bgs.²⁵ The soil extraction method was similar to the approach used by Higgins *et al.*²⁸ Briefly, a 500 mg soil sample was combined with 2.5 mL of 0.1%

ammonium hydroxide (NH_4OH) in methanol and mixed. The mixture was centrifuged and the supernatant transferred. The extraction was repeated two more time. The combined extract was evaporated to dryness and then reconstituted in 1.5 mL of 0.1% acetic acid in methanol. Cleanup was achieved by passing the extract through ENVI-CARB. For Task 2.2. soil and sediment extracts and TOP assay samples were analyzed by LC-MS/MS as described in Houtz et al.¹

For the TOP assay, groundwater was combined with 60 mM potassium persulfate in 0.125 M NaOH followed by heating for 6 h at 85°C. Soil and sediment extracts were evaporated to dryness with N_2 before adding 6 mL of 60 mM persulfate and 0.125 M NaOH before heating for 6 h at 85°C. After reaction, all samples were neutralized with concentrated HCl and amended with methanol and internal standards prior to analysis by LC-MS/MS.¹ for treatment of the TOP assay day, the total molar concentration of PFCAs produced by oxidation was reported as total PFAS concentrations.

For **Task 2.3**, our team members participated in a field study at Ellsworth AFB In collaboration with Chris Higgins, the PI on SERDP project ER-2126, in which the PFAS occurrence and distribution was described. Individual PFASs and the TOP assay precursors were mapped.¹⁶

Task 3: Biotransformation of AFFF PFASs and Impact on TCE Biotransformation

Task 3.1 and 3.2 Anaerobic Microcosms with Ansul AFFF

Anaerobic microcosms were constructed to test for potential AFFF biotransformation under different electron acceptor regimes that are comparable to those that occur in deep groundwater or sediments.

Chemicals and Standards. The Ansul AFFF used in this study contained 4:2, 6:2, and 8:2 FTAAoS as the primary PFASs and was obtained from the US Air Force.⁶ Unlabeled and stable-isotope standards for PFCAs, PFSAs, and 6:2 FTSA were purchased from Wellington Laboratories (Guelph, Ontario, CA), and Zonyl FSA was purchased from Sigma-Aldrich. Zonyl FSA is a proprietary mixture containing n:2 (n = 6, 8, 10) fluorotelomer thioether propionate (FTTPA). High performance liquid chromatography (HPLC)-grade water and methanol were purchased from Fisher Scientific. All other chemicals and solvents were purchased from either Fisher Scientific or Sigma-Aldrich at the highest possible purity.

Microcosm Setup. To understand the biotransformation of PFASs in AFFF under anaerobic conditions, microcosms were constructed with pristine or AFFF contaminated solids under sulfate-reducing conditions. Pristine solids were collected from the sediment of a creek on UC Berkeley campus and AFFF contaminated solids were from Ellsworth Air Force Base (South Dakota). The initial 6:2 FTAAoS concentration in the microcosms was approximately 20 μM . Ten g of pristine solids and five g of AFFF-contaminated solids were used as microbial inocula.

Triplicate live and autoclaved microcosms were prepared in 160 mL glass serum bottles with a N_2/CO_2 headspace (80/20 (v/v) and 50 mL basal medium containing cysteine sulfide as reducing reagent and resazurin as redox indicator.⁴ Also, 50 mM of sodium sulfate was amended as electron acceptor along with 50 μL neat AFFF and 1.5 mM DGBE, the primary organic solvent in the AFFF formulation) as electron donor and carbon source for the microcosms at the

beginning of incubations. Autoclaved controls were constructed using autoclaved soils and media-only controls consisted of media, but no solids. All microcosms were mixed by gentle swirling to avoid the formation of foams prior to incubation at 30 °C in the dark without shaking. Periodically, 50 mM sodium sulfate and 1.5 or 3 mM DGBE were amended to ensure that sufficient carbon source, electron donor and acceptor were present in the microcosms during the incubation period. Slurry samples were analyzed for transformation products by LC-MS/MS.

Analysis. Analytes including 6:2 FTTAoS, 6:2 FTTPA, 6:2 FTSA, PFCAAs, and PFSAs were analyzed by LC-MS/MS.¹ Concentrations of FTTPAs in Zonyl FSA were estimated assuming an equimolar response to that of the corresponding FTSAAs. The TOP assay was used to quantify the total amount of precursors in the microcosms.¹ Samples were dried and then reconstituted in a solution containing 116 mM sodium hydroxide and 51 mM potassium persulfate and incubated for 12 h at 85 °C (water bath). The reacted solutions were then diluted, vortexed, and analyzed for total PFCA concentrations using LC-MS/MS.

Identifying transformation products. Samples were analyzed with both high-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. FT-ICR analysis was performed with a Thermo Finnigan LTQ FT Ultra High Performance Mass Spectrometer (Thermo Finnigan, San Jose, CA) in the UC Berkeley QB3/Chemistry mass spectrometry facility. Full-scan MS data were acquired in the negative electrospray ionization mode over the m/z range of 100 - 1000 with a resolution setting of 100,000 (at m/z = 400). Xcalibur™ software (version 2.0.7 SP1, Thermo Scientific, Waltham, MA) was used to extract full scan mass spectra from FT-ICR.

Task 3.1 and 3.2 Aerobic Microcosms with Ansul AFFF⁴

Chemicals and Standards. Ansul AFFF was obtained from the Air Force⁶ and contained 4:2, 6:2, and 8:2 FTTAoS, as well as the sulfone form of 6:2 FTTAoS along with 220 g/L DGBE, which corresponded to 80% of the total organic carbon in the AFFF. A commercial source of 6:2 FTTAoS⁶ was used as a standard reference material for quantitative analysis.

Microcosm Setup. Microcosms were constructed using 250 mL glass bottles, 60 mL of a 30 mM bicarbonate-buffered mineral medium, 60 µL of Ansul AFFF, and 5 g of soil from Ellsworth Air Force Base. Sterile controls consisted of autoclaved soil amended with medium along with 0.5 g/L sodium azide and 60 µL AFFF. Soil-free media controls contained only sterile medium, sodium azide, and Ansul AFFF. All microcosms were run in triplicate while shaking at 100 rpm at 30° C for 60 d. Headspace and slurry samples were taken with syringes. Headspace oxygen was maintained at 15 and 25% (v/v) by adding pure oxygen periodically. Live microcosms received two additions (60 µL) of Ansul AFFF on days 0 and 18. Autoclaved and media-only controls received only 1 Ansul AFFF addition. On day 40, additional carbon in the form of DGBE (300 mg/L) was amended to the live microcosms. No microbial activity was observed in the autoclaved and media-only controls, as determined by the absence of organic carbon and oxygen consumption.

Analysis. The concentrations of PFCAAs; 4:2, 6:2, and 8:2 FTSAAs; saturated fluorotelomer carboxylates (FTCAs), and unsaturated fluorotelomer carboxylates (FTUCAs) acids were determined by LC-MS/MS.^{1,46} Slurry samples also were analyzed by the TOP assay. Briefly, 100 µL of slurry was combined with 3 mL HPLC-grade water, and 3 mL of 120 mM potassium

persulfate in 0.25 M NaOH. The reaction was then carried out at 85°C for 12 h. Prior to LC-MS/MS analysis, the reaction media was neutralized and then combined with 1 mL methanol. Headspace oxygen was measured by gas chromatography flame ionization detection. Dissolved organic carbon was measured using a Shimadzu TOC-V analyzer.

Task 3.3: Impact of AFFF on TCE Biotransformation⁵

AFFF, DGBE, and TCE Microcosms. Various AFFF formulations, PFASs, and ethylene glycol were amended to the growth medium of a *D. mccartyi* -containing enrichment culture to determine the impact on dechlorination, fermentation, and methanogenesis as described in Harding Marjanovic et al.⁵ Three characterized AFFFs (3M, National Foam, and Ansul) were used for the microcosm experiments. Commercial source materials containing FTTAoS, FTSAbs, FTSAm, perfluoroalkyl sulfonamido amines (PFSaAm), and perfluoroalkyl sulfonamide amino carboxylates (PFSaAmA) were obtained from the Fire Fighting Coalition,⁶ and the concentrations of 6:2 FTTAoS, 6:2 FTSAb, and 6:2 FTSAm in various stock solutions were previously determined by Backe et al.² All other standards were purchased from Wellington Laboratories (Guelph, Ontario, CA), while TCE and DGBE were purchased from Sigma Aldrich (St. Louis, MO).

All microcosm experiments were conducted in 160 mL glass serum bottles with 100 mL of a reduced basal medium with a vitamin solution containing 100 µg/L vitamin B₁₂ and a N₂/CO₂ (90:10) headspace. Serum bottles received 20 to 30 µmoles of neat TCE equilibrated 24 h prior to adding 5% (vol/vol) of an active *D. mccartyi*-containing enrichment culture.²⁰

AFFF Amendment. For experiments with AFFF and TCE, the experiments were conducted in triplicate with 300 µL of either Ansul, 3M, or National Foam AFFF were added to the growth medium and equilibrated for 24 h before inoculating with 5 mL of *D. mccartyi*. Sterile controls consisted of adding a previously-autoclaved culture to bottles containing AFFF and TCE and a second set that contained only inoculum-free medium and no microbial culture. Live controls consisted of growth medium, 2 mmoles lactate, 25-30 µmoles TCE, but no AFFF. At each sampling point, 1-1.5 mL of culture was removed and of that volume, 200 µL was diluted with 200 µL methanol for LC-MS/MS analysis

DGBE Amendment. Triplicate 160 mL bottles contained 100 mL growth medium, 25 µmoles TCE, and either 1) 250 µmoles DGBE, 2) 250 µmoles sterile DGBE stock solution and 2 mmoles lactate, or 3) 2 mmoles lactate. The DGBE concentration was selected to model the concentration associated with the 300 µL volume of AFFF as described above. Bottles initially amended with DGBE received additional DGBE amendments on days 28, 36, 92, and 118. Bottles with lactate + DGBE received one additional DGBE on day 92. All bottles received additional TCE (25 µmoles) on day 92. All DGBE amendments were periodically sampled by removing 1-1.5 mL of culture.

PFAS and AFFF Amendments. Triplicate 60 mL bottles were prepared with growth medium (50 mL) with 3% (vol/vol) of the *D. mccartyi* culture in a N₂/CO₂ (90:10) headspace along with 2 mmoles lactate, 25 µmoles TCE, and either 18 mg/L ethylene glycol, 12 mg/L 1-propanol, 45 mg/L FTTAoS, 16 mg/L 6:2 FTSAb, or 32 mg/L 6:2 FTSAm. Controls consisted of lactate-only bottles. Concentrations of the PFASs were selected to mirror those in the AFFF amendments described above. All bottles were incubated in the dark for five days at 34 °C.

To further assess the impact of PFSAs on TCE dechlorination, three different PFSA amendments were tested with enrichment cultures fed with 2 mmoles lactate and 22 μ moles TCE. These amendments contained 7.3, 22, and 36.7 mg/L of PFBS, PFHxS, and PFOS each, yielding three tested conditions with total PFSA concentrations of 22, 66, and 110 mg/L, respectively.⁴⁷ In addition, bottles were constructed with the addition of a suite of PFCAs and PFSAs at concentrations of 2, 6, and 10 mg/L of each PFAS, yielding total PFAS concentrations of 22, 66, and 110 mg/L.⁴⁷ The methanol in the stock solutions was removed by evaporation prior to adding anaerobic growth medium, 1 mmole lactate, and 22 μ moles TCE. The mixture was inoculated with 1.5 mL *D. mccartyi* enrichment culture and incubating for seven days at 34 °C. Controls consisted of bottles that received all materials except the mixture of perfluorinated acids.

Analytical Methods. Chloroethene, methane, and hydrogen were quantified by gas chromatography flame ionization detection,²⁰ PFASs were quantified by LC-MS/MS;¹ and organic acids (i.e., lactate, acetate, butyrate, and propionate) were quantified by HPLC.¹⁹ The 16S ribosomal deoxyribonucleic acid (rDNA) genes of *D. mccartyi* genes were quantified by polymerase chain reaction.¹⁹

Task 4 Sorption of Cationic and Zwitterionic PFASs to Soils and Sediments

Soil Sample Collection and Characterization. Six blank soils (Soils 1-6) were selected from an archived soil collection at Oregon State University that had pH values of 5.0-5.5, but covered a range of soil characteristics, including organic carbon, CEC, and anion exchange capacity (AEC) (Table 1). Soils were sieved to < 2 mm and air dried prior to homogenization with a mortar and pestle before use in isotherm experiments.

Table 1. Soil properties used in sorption experiments.³

Soil #	pH	% OC ^a	% N ^a	C/N Ratio	CEC ^b (meq/100g)	AEC ^d (meq/100g)
1	5.1	1.5	0.13	13	8.7	0.20
2	5.5	0.12	0.02	6.8	19	0.58
3	5.1	2.3	0.20	13	6.8	1.2
4	5.0	7.7	0.43	21	7.1	2.2
5	5.0	1.0 \pm 0.19 ^f	0.084 \pm 0.0087	12 \pm 1.1	9.5 \pm 1.9	0.28 ^g
6	5.2	0.098	0.004	30	35	0.33

^aPercent organic carbon (% OC) and nitrogen (N) as determined by the method of Goni et al.⁴⁸ ^bCation exchange capacity measured by summation method, pH 7. ^dAnion exchange capacity at native soil pH (using water).

Batch Sorption Experiments. All isotherms, with the exception of the preliminary experiment involving a commercial product, used National Foam AFFF that contained anionic, zwitterionic, and cationic PFASs. The pKas for each chemical form (Fig. 2) were derived by comparing the molecules to model compounds in the literature.

A 21-point isotherm was constructed using Soil 1, with initial aqueous phase concentrations (C_w) of 1,000 – 138,000,000 ng/L for the 6:2 FTSA_B. Eleven-point isotherms were constructed using Soils 2-6 and two additional isotherms were constructed with Soil 1 that was titrated to pH 4 and 7. For the 11-point isotherms, C_w ranged from 1,000 to 250,000 ng/L for the 6:2 FTSA_B. The upper-end C_w for the 21-point and the 11-point isotherms were selected based on the 3% AFFF in water used in firefighting and the mid-range concentration was selected to represent the 6:2 FTSA concentration in AFFF-impacted groundwater (8,900 – 220,000 ng/L),² respectively. Replicates, which consisted of homogenized soil spiked with the same volume of National Foam AFFF stock solution, were created for 6:2 FTSA_B C_w of 5,000 ng/L (n=4), 60,000 ng/L (n=3), and 138,000,000 ng/L (n=3). All other data are derived from individual reactors.

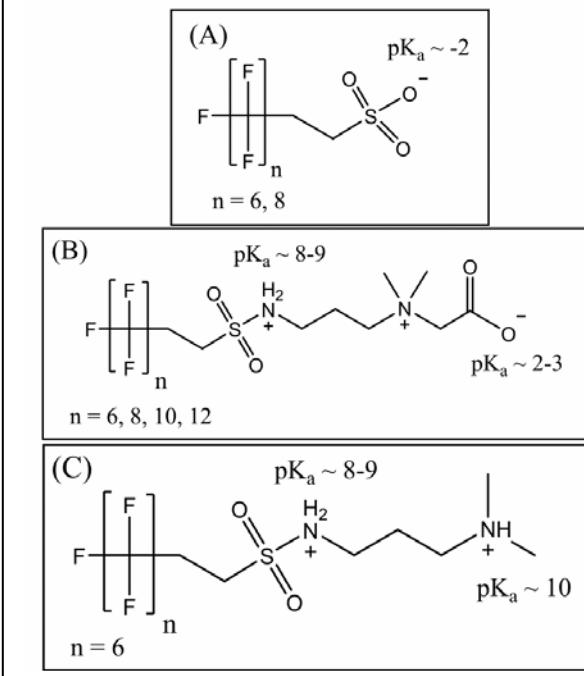
Homogenized soil was weighed (1.00 ± 0.02 g) into each 50 mL polypropylene centrifuge tube and autoclaved for 40 min at 121 °C with a 20-min drying time (Consolidated Sterilizer Systems Model SSR-2A-ADVPB, Allston, MA). Vials contained the autoclaved soil and 10 mL 0.5 mM calcium chloride,²³ to which the diluted National Foam AFFF was spiked. Positive controls consisted of National Foam AFFF prepared at the concentration of the replicates but no soil. One negative control, consisting of the autoclaved soil and 0.5 mM calcium chloride was prepared with each isotherm. Vials were allowed to equilibrate for 24 h on a wrist action shaker at 10° rotation (Burrell Corporation, Model 75, Pittsburg, PA).⁴⁹

Following equilibration, batch reactors were centrifuged at 2808 G for 20 min (Eppendorf, Model 5810R, Hauppauge, NY), and the supernatant was decanted into a separate 15 mL centrifuge tube. The soil was transferred into a separate 50 mL centrifuge tube using deionized water. The equilibrated vial phase (hereafter referred to as vial phase) and the aqueous phase were stored at -20 °C. The soil was freeze dried for 48 h and subsequently stored in the dark at 22 °C until extraction.

Aqueous Sample Preparation and Analysis. After equilibration, the aqueous phase was diluted 1-5,520-fold in groundwater surrogate for the Soil 1 native pH isotherm and 1-10-fold for the remaining isotherms (Soils 2-6, pH 4, pH 7). The diluted aqueous phase was extracted using a micro liquid-liquid extraction as described elsewhere with slight modifications.²

Mass Balance. Mass balance experiments consisted of the 60,000 ng/L 6:2 FTSA_B (C_w = 4) conditions with Soil 1 that were set up and treated in the same manner as the isotherms. Mass balance experiments were conducted using the aqueous phase extraction and a soil extraction

Fig 2. Chemical structures and charged states of PFASs investigated: FTSA_S (A), FTSA_Bs (B), and 6:2 FTSA_{Am} (C) in National Foam AFFF.



typically used for cationic and zwitterionic PFASs.^{1,29,30} The vial and soil phases were extracted using 1.5 mM NH₄OH in methanol as described elsewhere.¹ Preliminary experiments indicated poor recovery of the zwitterionic 6:2 FTsAB and the cationic 6:2 FTsAm (data not shown) when soil and analytes were equilibrated in an aqueous phase (and not methanolic spikes) and extracted using relatively mild conditions (1.5 mM NH₄OH in methanol,¹ 20 mM sodium hydroxide (NaOH) in methanol,³⁰ and 1% acetic acid in methanol.²⁹ Soil extraction methods for cationic, non-fluorinated surfactants typically use either 0.5 M⁵⁰ or 1 M HCl^{34,51,52} in methanol. The 1 M HCl in methanol extraction conditions proved to be problematic (unpublished data). Therefore, 0.5 M HCl in methanol was used in all subsequent soil extractions.

Mass balance experiments were repeated using the acidic methanol extraction for the vial and soil phases. Recoveries improved significantly for the 6:2 FTsAB and the 6:2 FTsAm (95-105% recovery). Mass balance experiments also indicated that sorption to the vial walls was minimal for all analytes (unpublished data). Therefore, only the aqueous and soil phases were extracted for each vial (not the vial itself). Due to the high concentration of the stock solution and subsequent variability of the mass of analyte added to each vial, mass balance on each vial was not feasible. Since separate mass balance studies with a known mass of analyte added achieved mass balance, mass balance was assumed in all vials. The analytes were quantified by LC-MS/MS.²

Data Treatment. The fraction of pore water following equilibration was determined so soil phase concentrations for each analyte could be adjusted to account for the mass of each analyte associated with remaining pore water. Sorption isotherms were fitted using the following Freundlich isotherm model equations:

$$C_s = K_f C_w^n \quad (\text{Eqn 1})$$

$$\log C_s = n \log C_w + \log K_f \quad (\text{Eqn 2})$$

where K_f is the Freundlich sorption coefficient and n is a measure of nonlinearity and represents the free energy associated with adding more sorbate to the sorbent.⁵³ K_f values were converted to the concentration-specific K_d values to enable comparisons between isotherms and across other sorption studies for other PFASs. To incorporate the greatest amount of uncertainty, the K_d values from the 60,000 ng/L C_w are used in the discussion below.

RESULTS AND DISCUSSION

Task 1: Characterize AFFF formulations

Information, including figures and tables, contained in this section is documented in the following published papers:

Place, B., Field, JA. **2012**. Identification of novel fluorochemicals in aqueous film-forming foams (AFFF) used by the US military. *Environmental Science and Technology*, 46, 7120-7127.

Houtz, E., Higgins, C., Field, J. and Sedlak, D. **2013**. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environmental Science and Technology*, 47, 9342-9349.

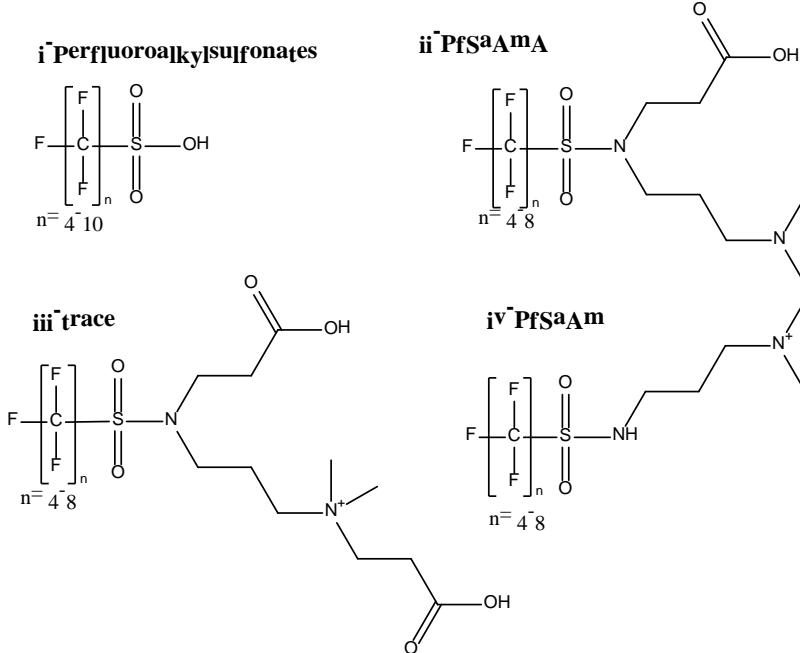
Barzen-Hanson, K., Field, J.A. **2015**. Discovery and implications of C2 and C3 perfluoroalkyl sulfonates in aqueous film forming foams (AFFF) and groundwater. *Environmental Science and Technology Letters*, 2, 95-99. Won Editor's Choice and Environmental Science and Technology Letters Paper of the Year award.

Barzen-Hanson, K., Simon, R., Choyke, S., Oetjen, K., McAlees, A., Riddell, N., McCrindle, R., Ferguson, P., Higgins, C., Field, J. **2017**. Discovery of 40 classes of per- and polyfluoroalkyl substances in historical aqueous film-forming foams (AFFF) and AFFF-impacted groundwater. *Environmental Science and Technology*, 51, 2047-2057.

Task 1.1 Define PFAS Composition of AFFFs

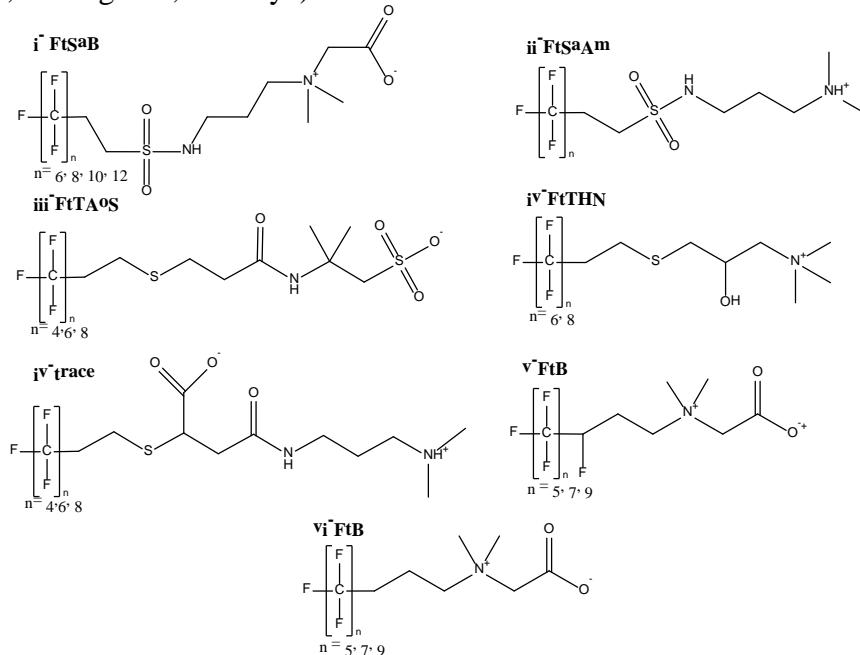
For **Task 1.1**, a total of 11 classes of PFASs were initially identified in AFFF formulations stockpiled at US military sites (Fig 3 and 4).¹

Fig 3. Major and trace level classes of PFASs identified in ECF-based AFFFs.¹



All the PFASs shown in Fig. 3 are derived from electrofluorination (ECF) chemistry by 3M. In addition to PFSAs, which were expected, three additional zwitterionic and cationic classes were identified (Fig 3). Examination of the new structures and their fluorinated chain lengths reveal their potential to form PFSAs. The PFASs in 3M AFFF all had perfluorinated chains directly bonded to a sulfur atom, which indicate the potential to degrade to PFASs. Six major classes and one minor class were identified in the fluorotelomer-based AFFFs (Fig 4). Only the FTAAoS (Fig 5iii)^{10,54} and (FTSaB (Fig 5i)⁵⁵ have since been identified by others in AFFFs.^{55,56} The polyfluorinated forms contained the characteristic fluorotelomer-based chain with an even number of fluorinated carbons bonded to two carbons bearing hydrogens. In terms of nomenclature for Fluorotelomer-based substances, ‘6:2’ refers to six perfluorinated carbons and two methylene carbons. For example, 6:2 FTSA represents $\text{F}_3\text{C}(\text{CF}_2)_5\text{CH}_2\text{CH}_2\text{SO}_3^-$.

Fig 4. Major classes of polyfluorinated identified in fluorotelomer-based (Ansul, National Foam, Angus, Chemguard, Buckeye) AFFFs.¹



Quantitative analytical method for groundwater. Once the PFASs were identified in the 3M and fluorotelomer-based AFFFs, the next step was to quantify the individual PFASs in the AFFF formulations. However, at the time these PFASs were identified, analytical methodology for these chemicals had not been developed. It is for this reason that a new analytical method was developed as part of Task 1 to quantify these newly-identified PFASs.

The final method briefly consists of adding salt and then acidifying a small (3 mL) volume of groundwater that is then extracted with ethyl acetate. This approach was selected over more conventional water sample extractions for PFASs, such as EPA method 537, because it avoids the use of solid phase extraction, thus minimizing the potential for sample contamination and analyte loss and the generation of solid and liquid waste. The method is efficient and cost effective because it involves minimal handling. For example, once the ethyl acetate extract is generated, 0.9 mL or 60% of the 1 mL extract is injected directly into the LC-MS/MS system

without further sample cleanup. In contrast, analytical methods based on solid phase extraction typically inject only 10-20 μ L, which is a much smaller fraction of the extract generated, thus wasting most of the time and effort required to generate the extract. The analytical method is novel because the PFASs in the 900 μ L extract are isolated/concentrated from the ethyl acetate by polar mechanisms on a combination of small guard columns (silica followed by an amino propyl). The analytes trapped by small guard columns, which operate by ion exchange, are then eluted with ammonium acetate buffer/methanol of sufficiently low elutropic strength so that they are then focused on the head of the C18 analytical column where the final analyte separation takes place. An example of the resulting separation is shown in Fig 5.

Application of Analytical Method to PFASs in AFFF. AFFF formulations were analyzed

by LC-MS/MS in order to determine the proportions of the chemical classes for each AFFF.² The 3M formulations contained only ECF-based PFASs (Fig 3) and the mixtures were predominantly C4-C10 PFSAs with lesser levels of the C4-C8 PFCAs (Table 2). The ratio of PFSAs to PFCAs in the 3M formulations was \sim 20:1 (Table 2), with PFOS as the primary component. The composition of 3M AFFF formulations changed around 1993 to contain greater proportions of PFSaAm and PFSaAmA classes that are primarily C6 in chain length (Table 2). However, it is important to note that we only obtained AFFF formulations dating back to 1989. Discussions with the Fire Fighting Foam Coalition, who provided a letter of support for this SERDP project, indicate that 3M revealed in meetings that potentially higher levels of PFCAs were used in their older AFFF formulations. Efforts to obtain older AFFF formulations were unsuccessful.

Only polyfluoroalkyl substances were quantified in fluorotelomer-based AFFFs varies by manufacturer (Table 3). PFCAs were not detected in the telomer-based AFFF formulations (Table 3) at the dilution levels employed (1:100,000 – 1:1,000,000). The FTSAs were only quantified at relatively low levels in telomer-based AFFFs, which indicates that FTSAs in groundwater are likely due to the biotransformation of higher molecular weight precursors, including FTtaoS, FTSaB, and FTSaAms (Fig. 2). Within the FTSaB class, the polyfluorinated chain lengths ranged from 6:2 to 12:2. The longer chained 8:2, 10:2, and 12:2 FTSaBs are potentially of interest since the structures indicate the potential to form long chain (\geq C8) PFCAs.

Fig 5. Typical chromatograms of zwitterionic, cationic, and anionic, and analytes detected in groundwater samples (unspiked) from military bases.²

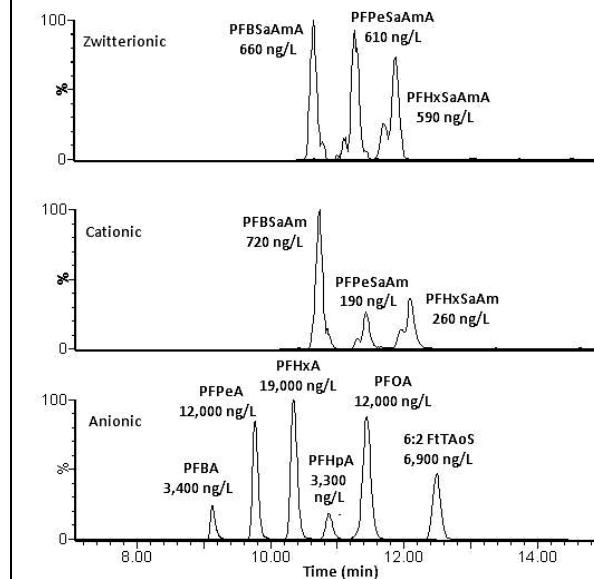


Table 2. Concentrations in mg/L of newly-identified and legacy PFASs in 3M AFFF formulation by year of manufacturing. PFCAs above C8 (PFOA) were not detected.²

Analyte	1989 mg/L	1989 mg/L	1993 mg/L	1993 mg/L	1998 mg/L	2001 mg/L
PFBSaAm ^a	2.4	1.9	83	100	95	61
PFPeSaAm ^a	4.7	1.1	71	97	89	62
PFHxSaAm ^a	120	120	470	660	690	550
PFOSaAm ^a	2.0	0.4	24	17	24	15
PFBSaAmA ^a	<LOD	<LOD	160	130	110	130
PFPeSaAmA ^a	<LOD	<LOD	100	71	86	110
PFHxSaAmA ^a	ND	<LOD	630	520	530	800
PFOSaAmA ^a	<LOD	<LOD	13	14	16	13
PFBS	240	240	160	110	150	190
PFHxS	1,100	1,000	780	520	670	800
PFHpS	160	140	75	50	63	97
PFOS	6,000	5,800	4,600	3,500	3,900	5,200
PFDS	7.9	10	6.9	3.5	3.6	9.5
PFBA	15	14	9.4	10	17	20
PFPeA	22	23	15	15	19	34
PFHxA	160	150	88	100	110	160
PFHpA	33	36	21	14	22	34
PFOA	84	77	58	54	60	120

<LOD = less than the limit of detection

Table 3. Concentrations in mg/L of polyfluorinated chemicals in fluorotelomer-based AFFFs by manufacturer.²

	National Foam	Chemguard	Ansul	Buckeye Fire Equipment	Angus	Fire Service Plus
	2003	2010	2005	2009	2002	NR
Analyte	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
4:2 FTTAoS	<LOD	<LOD	26	<LOD	25	<LOD
6:2 FTTAoS	<LOD	11,000	6,100	<LOD	4,900	<LOD
8:2 FTTAoS	<LOD	24	1,100	<LOD	170	<LOD
6:2 FTTHN	<LOD	<LOD	<LOD	<LOD	2,200	<LOD
6:2 FTSaB	4,600	<LOD	<LOD	<LOD	<LOD	4,800
8:2 FTSAa	540	<LOD	<LOD	<LOD	<LOD	1,800
10:2 FTSaB	450	<LOD	<LOD	<LOD	<LOD	830
12:2 FTSaB	210	<LOD	<LOD	<LOD	<LOD	430
6:2 FTSaAm	2,100	<LOD	<LOD	<LOD	<LOD	3,400
8:2 FTSaAm	450	<LOD	<LOD	<LOD	<LOD	720
5:1:2 FTB	<LOD	<LOD	<LOD	2,000	<LOD	<LOD
7:1:2 FTB	<LOD	<LOD	<LOD	4,700	<LOD	<LOD
9:1:2 FTB	ND	<LOD	<LOD	1,900	<LOD	<LOD
5:3 FTB	ND	<LOD	<LOD	530	<LOD	<LOD
7:3 FTB	<LOD	<LOD	<LOD	610	<LOD	<LOD
9:3 FTB	<LOD	<LOD	<LOD	430	<LOD	<LOD
4:2 FTSA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6:2 FTSA	42	<LOD	<LOD	<LOD	<LOD	53
8:2 FTSA	19	<LOD	<LOD	<LOD	<LOD	56

Additional PFASs Discovered in AFFF and Groundwater. Two separate additional studies were conducted after the first round of PFAS discovery in 2010.⁶ Using high mass accuracy QTOF-MS, perfluoroethane sulfonate (PFEtS) and perfluoropropane (PFPrS) sulfonate were identified in AFFF. The analytical method developed for the PFASs found in AFFF and groundwater in 2010 was then adapted to quantify PFEtS and PFPrS in AFFF and groundwater.⁴⁰ Concentrations of PFEtS and PFPrS ranged from 7-13 mg/L and 120-270 mg/L, respectively, in 3M AFFFs. Eleven groundwaters collected from AFFF-contaminated sites gave PFEtS at concentrations of 11-7, 500 ng/L and PFPrS at concentrations from 19-63,000 ng/L. These ultra-short chained forms are characterized by high aqueous solubility and mobility in the subsurface, which will likely make them difficult to remove by conventional activated carbon treatment.

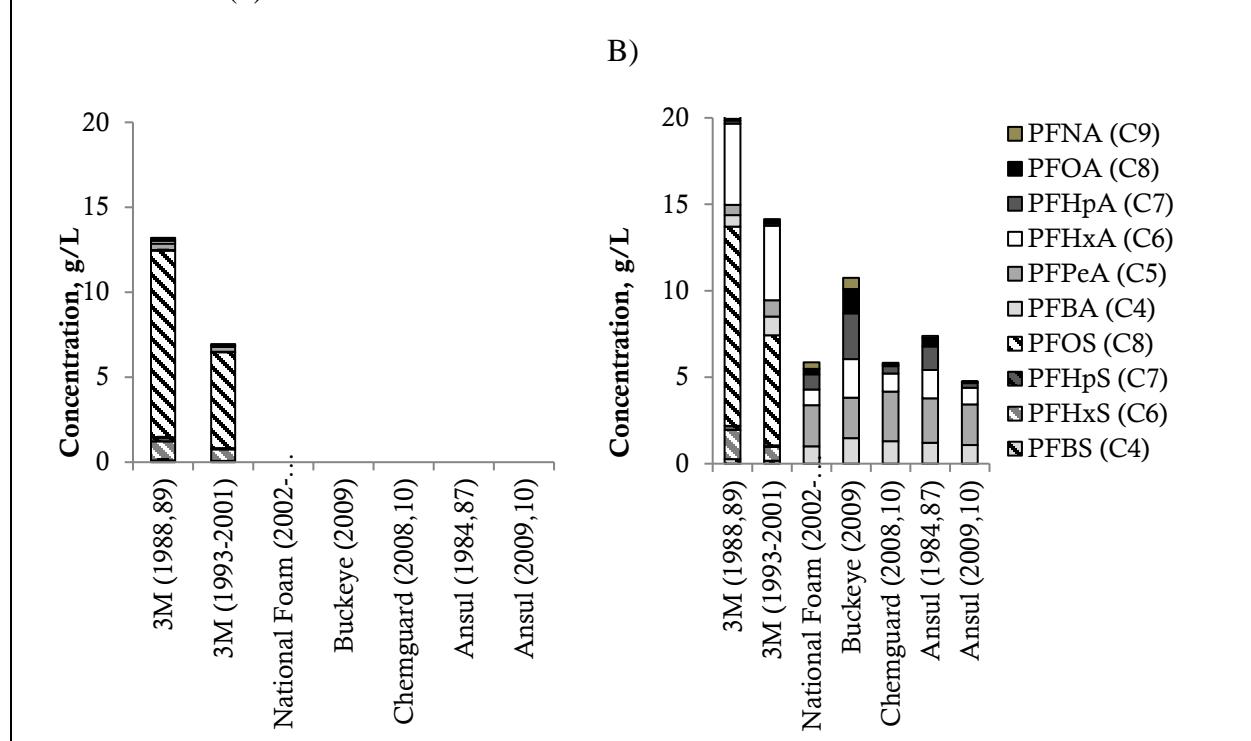
The final phase of discovery, was more thorough in the number and type of PFASs identified.⁴¹ The result is that 40 classes of novel anionic, zwitterionic, and cationic PFASs were discovered. In addition, 17 previously-reported classes were observed for the first time in AFFF and AFFF-impacted groundwater. With the assistance of Wellington laboratories, all 57 classes were

assigned an acronym. Thirty four of the 40 newly-identified PFAS classes derive from the 3M ECF-based process. Of the 13 classes identified only in AFFF impacted groundwater, 11 were ECF-derived, while the two remaining classes are associated with fluorotelomer-based chemistry.⁴¹ The frequency of occurrence and concentrations are not determined for this study and will be part of future work conducted under ESTCP ER-201633 and SERDP ER-2720. With the identification of these additional classes, it may now be possible to close the mass balance on highly-fluorinated substances in US military groundwater. Additional work is needed to compare the estimated concentrations of the PFASs to other indicators, such as total fluorine by PIGE and the TOP assay. Given the structures, most are water-soluble, mobile, and contribute to the total mass of highly-fluorinated substances to be removed from potential drinking water sources. Work is underway with AECOM under the AFCEC Contract FA8903-12-C0005 to determine the removal of the newly-identified PFASs from Wurtsmith groundwater by granulated activated carbon.

Task 1.2 Total Oxidizable Precursor Assay

For **Task 1.2**, the precursors present in AFFF formulations were found to convert only to perfluorinated carboxylates. Analysis before and after persulfate treatment gave significant increases in the concentrations of the PFCAs in all AFFF formulations (Fig. 6). Concentrations

Fig 6. Concentrations of PFSAs and PFCAs in AFFF formulations analyzed before (a) and after oxidation (b).¹



of PFSAs were unchanged by oxidation (compare Fig 6a and 6b). In the 3M formulations, PFHxA was the main PFCA produced during oxidation, which indicates a predominance of C6 chain-length precursors in 3M formulations. Chemguard, Ansul, National Foam, and Buckeye AFFF formulations also produced relatively high concentrations of PFHxA, as well as other short-chain PFCAs upon oxidation. Ansul formulations from the mid-1980s and National Foam

and Buckeye formulations produced PFOA upon oxidation. Neither the 3M formulation nor the AFFFs from Ansul and Chemguard consistently generated significant amounts of PFOA upon oxidation, which suggests that these formulations contain precursors with less than eight perfluorinated carbons.

In addition to the AFFF formulations, reference materials including Forafac 1157 and FTTAoS, which are found in the AFFFs sold by National Foam and Ansul, respectively, were analyzed by the TOP assay. A mixed suite of PFCAs evolved with complete disappearance of the monitored parent precursor compounds. The molar profiles of the reference materials were consistent with the PFCA product profile of corresponding AFFF formulations upon oxidation. The concentrations of PFCAs evolved in an AFFF formulation after oxidation is used as a conservative quantitative estimate for the concentration of precursor compounds initially present in the AFFF formulations because recovery of standards as PFCAs was variable and significantly less than 100 %.

Task 2: Characterize Groundwater, Sediment and Soil at AFFF-Contaminated Sites

The information, including figures and tables, contained in this section is documented in the following published papers:

Backe, W.J. and Field, J.A. **2012**. Is SPE necessary for environmental analysis? A quantitative comparison of matrix effects from large-volume injection and solid-phase extraction based methods. *Environmental Science and Technology*, 46, 6750–6758.

Houtz, E., Higgins, C., Field, J. and Sedlak, D. **2013**. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environmental Science and Technology*, 47, 9342-9349.

Backe, W.J., Christensen, K.E., and Field, J.A. **2013**. Newly-identified cationic, anionic, and zwitterionic fluorinated chemicals in groundwater at US military bases by large volume injection HPLC – MS/MS. *Environmental Science and Technology*, 47, 5226-5234.

McGuire, M.E., Schaefer, C., Richards, T., Backe, W.J., Field, J.A., Houtz, E., Sedlak, D.S., Guelfo, J.L., Wunsch, A., Higgins, C.P. **2014**. Evidence of Remediation-Induced Alteration of Subsurface Poly- and Perfluoroalkyl Substance Distribution at a Former Firefighter Training Area. *Environmental Science and Technology*, 48, 6644-6652.

Task 2.1 Analysis of Groundwater from Field Sites

Under **Task 2.1**, the new analytical method developed in Task 1.1 was applied to field site groundwater from Ellsworth and Randolph AFBs. The method detection limits ranged from 0.71 ng/L to 67 ng/L and whole-method accuracy ranged from 96±8.4% to 106±9.1% for analytes with authentic analytical standards.² For analytes without authentic analytical standards, whole-method accuracy ranged from 78% to 144%. Method precision for all analytes was less than 15%. For some of the newly-identified analytes, the AFFF formulation represents the only source of the newly-identified PFASs. This means that the resulting data for the newly-identified PFASs will be semi-quantitative until high quality standards become available

commercially.

Application of the developed methodology to groundwater collected from Ellsworth AFB (Table 4) and Randolph AFB (Table 5) gave quantifiable concentrations of legacy (PFCAs, PFSAs, FTSAAs). Groundwater from Ellsworth AFB (Table 4), Randolph AFB (Table 5), and sites previously sampled including Wurtsmith AFB, Naval Air Station Fallon, and Tyndall AFB, gave quantifiable levels of 3M precursors (PFSaAm and PFSaAmA) as well as the FTTAoS (Appendix Table A7). However, at both the Ellsworth and Randolph sites, the concentrations of these precursors were significantly lower than those of PFSAs and PFCAs, which ranged in concentration up to a maximum of 360,000 ng/L (Table 4) at Ellsworth AFB and up to hundred and 170,000 ng/L at Randolph AFB (Table 5). These two data sets provide further evidence that the 6:2 FTSA occurs at concentrations similar to that of PFOA and PFOS. At Tyndall AFB, NAS Fallon, and Wurtsmith, the newly-identified chemicals (Appendix Table A7) were significantly lower than that of PFCAs, PFSAs, and FTSA, which ranged into the mg/L (10^6 ng/L) range¹⁰.

The groundwater data from Ellsworth and Randolph AFBs indicate a predominance of the C6 over that of the C8 chain lengths. The PFSAs can only originate or derive from 3M AFFF. However, AFFF formulation data indicates that PFHxS is lower in abundance than PFOS. Therefore, the predominance of PFHxS at field sites could be due to either biodegradation of C6-based precursors (PFSaAmA and PFSaAm), AFFF formulations enriched in PFHxS (prior to 1989), or differences in solubility and/or transport between the two homologs. This question was further examined as part of **Task 2.3** in an effort led by Chris Higgins, the PI of the SERDP project ER-2126. In contrast, PFCAs are not present in ECF-based AFFFs at levels comparable to PFSAs nor are they in telomer-based AFFF formulations at the dilutions used for the measurements. As such, PFCAs may arise from the biodegradation of fluorotelomer-based precursors or 3M AFFF formulations older than 1989 that are relatively enriched in PFCAs. Given the listing of Ansul AFFF on the QPL and the contracts held by Ansul in the 1980s, it is likely that the 6:2 FTTAoS may have biodegraded to form PFCAs.^{4,54} In Ansul AFFF formulations, the 6:2 FTTAoS is greater in abundance than the 8:2 form, so more C6 products would be expected and they would be expected to be only linear and not branched.

Table 4. Concentrations of PFASs quantified in groundwater from Ellsworth AFB.²

Analyte	Sample 1 ng/L	Sample 2 ng/L	Sample 3 ng/L	Sample 4 ng/L
4:2 FTTAoS ^b	<LOD	<LOD	210	490
6:2 FTTAoS	<LOD	<LOD	6,900	86
4:2 FTSA	370	6,500	7,500	11,000
6:2 FTSA	8,900	36,000	220,000	93,000
8:2 FTSA	120	58	370	180
PFBSaAm ^c	2.8 ^d	54	<LOD	<LOD
PFPeSaAm ^c	4.4 ^d	8.7	<LOD	<LOD
PFHxSaAm ^c	45	<LOD	<LOD	<LOD
PFBSaAmA ^c	<LOD	<LOD	<LOD	<LOD
PFPeSaAmA ^c	<LOD	<LOD	<LOD	<LOD
PFHxSaAmA ^c	<LOD	<LOD	<LOD	<LOD
PFBS	7,100	24,000	43,000	150,000
PFHxS	36,000	100,000	240,000	360,000
PFHpS	1,100	3,700	11,000	3,700
PFOS	19,000	15,000	78,000	19,000
PFDS	7.0	<LOD	<LOD	<LOD
PFBA	3,400	12,000	24,000	57,000
PFPeA	12,000	21,000	69,000	120,000
PFHxA	19,000	63,000	130,000	350,000
PFHpA	3,300	11,000	15,000	45,000
PFOA	12,000	35,000	51,000	220,000
PFNA	130	40	220	390
PFDA	17	<LOD	<3.1	6.5
PFUdA	<LOD	<LOD	<3.1	<LOD
PFDoA	<LOD	<LOD	<LOD	<LOD

< LOD = less than the limit of detection; ^aNo 8:2 FTTAoS, 6:2 FTTHN⁺, fluorotelomer sulfonamido betaines, fluorotelomer sulfonamido amines, nor fluorotelomer betaines were detected; ^b calculated assuming equal molar response to 6:2 FTTAoS (see main text); ^c calculated assuming equal molar response to PFOS; ^d concentration above limit of quantification but below the lowest calibration standard.

Table 5. Concentrations of PFASs quantified in groundwater from Randolph AFB.²

Analyte	Sample 1 ng/L	Sample 2 ng/L	Sample 3 ng/L	Sample 4 ng/L	Sample 5 ng/L	Sample 6 ng/L	Sample 7 ng/L	Sample 8 ng/L
4:2 FTTAoS ^b	<LOD							
6:2 FTTAoS	<LOD	<LOD	<LOD	68	<LOD	<LOD	<LOD	<LOD
4:2 FTSA	<LOD	5.2	<LOD	44	<LOD	100	160	99
6:2 FTSA	<LOD	1,400	210	860	3,500	15,000	3,900	37,000
8:2 FTSA	<LOD	660	660	66	1,200	2,300	620	1,400
PFBSaAm ^c	<LOD							
PFPeSaAm ^c	<LOD							
PFHxSaAm ^c	<LOD							
PFBSaAmA ^c	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.1 ^d	<LOD
PFPeSaAmA ^c	<LOD							
PFHxSaAmA ^c	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.0	<LOD
PFBS	12	1,500	640	7,300	2,900	2,800	17,000	24,000
PFHxS	81	11,000	10,000	9,800	18,000	17,000	74,000	170,000
PFHpS	<LOD	580	410	120	920	490	1,700	4,100
PFOS	88	15,000	23,000	4,000	29,000	20,000	44,000	65,000
PFDS	<LOD	33	<LOD	<LOD	16	<LOD	<LOD	26
PFBA	8.5	1,100	980	3,000	2,000	1,700	5,900	13,000
PFPeA	4.9 ^d	2,000	1,800	8,100	3,300	6,000	15,000	35,000
PFHxA	<4.7	5,400	2,400	12,000	11,000	7,700	29,000	99,000
PFHpA	<6.0	480	1,600	860	670	1,200	1,300	7,200
PFOA	8.6	890	2,500	840	1,700	3,700	3,000	57,000
PFNA	<LOD	56	680	15	110	110	130	400
PFDA	<3.1	8.0	19	<3.1	12	10	7.6	17
PFUdA	<LOD	3.7 ^d	5.2	<3.1	4.2 ^d	<3.1	<3.1	4.9 ^d
PFDoA	<LOD	<3.4	<LOD	<LOD	<LOD	<3.4	<LOD	<3.4

< LOD = less than the limit of detection; ^ano 8:2 FTTAoS, 6:2 FTHN+, fluorotelomer sulfonamido betaines, fluorotelomer

sulfonamido amines, nor fluorotelomer betaines were detected; ^b calculated assuming equal molar response to 6:2 FTTAoS; ^c

calculated assuming equal molar response to PFOS; ^d concentration above LOQ but below the lowest calibration standard.

Task 2.2 Analysis of Sediment/Soil from Field Sites

For **Task 2.2**, a total of 26 groundwaters, 16 soils, and 10 sediment samples from Ellsworth AFB were analyzed by the TOP assay for **Task 2.2**. After oxidation, the groundwaters all gave increased PFCA concentrations, indicating the presence of precursors. The C6 carboxylate increased to the greatest extent, indicating specifically the presence of C6 precursors in groundwater. A maximum of 50% of the total precursor concentrations in groundwater could be accounted for by individual, measured PFASs. This finding indicates that there are additional PFAS species present in groundwater that remained to be identified.

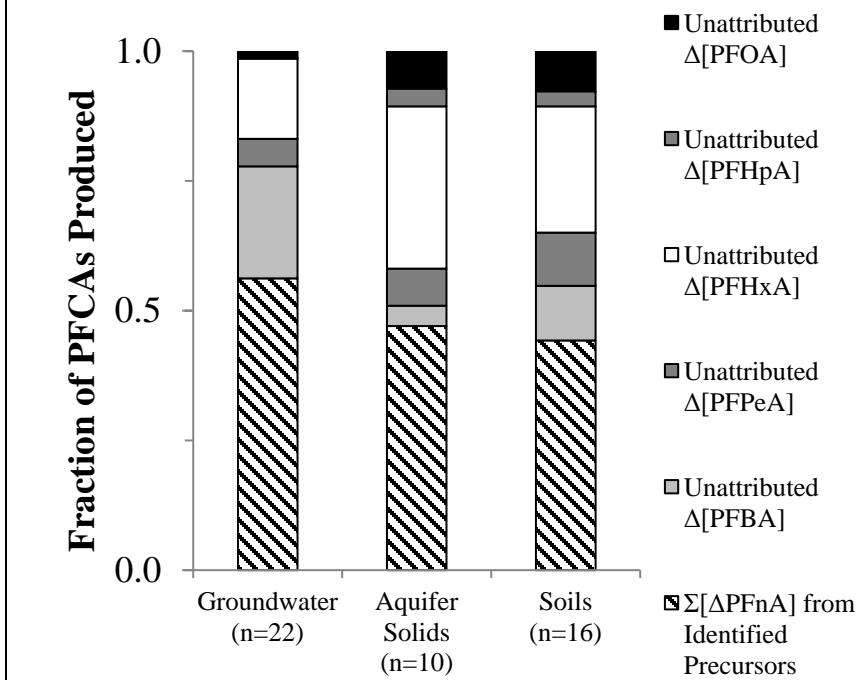
The PFASs that can be measured as individual components by LC-MS/MS account for ~ 50% of the precursors at this site (Fig. 7). Additional analytical work to identify precursors in soil and sediment is needed and will be part of SERDP ER-2720. As expected, oxidation of sediment/soil resulted in the production of only PFCAS and the formation of primarily the PFHxA indicated that precursors in soil/sediment are predominantly C6 compounds. Nearly all of the PFOA production was accounted for by the two C8 precursors measured, 8:2 FTSA and perfluoroocooctane

sulfonamide (FOSA). FOSA is a known intermediate in the degradation of sulfonamides¹⁴ and is present on soil and sediments at the site but not in groundwater..

When the concentrations of individual PFASs and total oxidizable precursors for groundwater and sediment/soil are examined together, several conclusions can be drawn. First, the newly-identified PFASs in AFFF formulations can be found in

groundwater and soil/sediment at the Ellsworth AFB site but only at trace levels. For this reason, it would appear that the precursor components of AFFF have undergone some level of biodegradation at this site or are sorbed to soil/sediments. Second, the predominance of C6 forms (e.g., PFHxA and PFHxS) at Ellsworth and Randolph AFBs (Table 4 and 5) and predominance of C6 precursors, as determined by the TOP assay, in groundwater, sediment, and soil are viewed as consistent with biodegradation of the C6 PFASs that are present in MilSpec AFFF

Fig 7. Fraction of unattributed PFCAs in groundwater, aquifer solids, and soils.¹



formulations or, alternatively, originate from AFFF formulations that differ in composition from those tested for this study.

As part of **Task 2.3** (analysis of spatial distribution and co-contamination with priority pollutants) this project collaborated with SERDP project number ER-2126, led by Dr. Christopher Higgins of the Colorado School of Mines.

Task 3: Biotransformation of AFFF PFASs and Impact on TCE Biotransformation

The information, including figures and tables, appear in the following publications:

Harding-Marjanovic, KD, Houtz, EF, Yi, S, Field, JA, Sedlak, D, and Alvarez-Cohen, L **2015**. Aerobic biotransformation of fluorotelomer thioether amido sulfonate (Lodyne) in AFFF-amended microcosms. *Environmental Science and Technology*, 49, 7666-7674.

Harding-Marjanovic, KD, Yi, S, Weathers, TS, Sharp, JO, Sedlak, D, and Alvarez-Cohen, L **2016**. Effects of aqueous film forming foams (AFFFs) on trichloroethene (TCE) dechlorination by a *Dehalococcoides mccartyi*, *Environmental Science and Technology*, 50, 3352-3361.

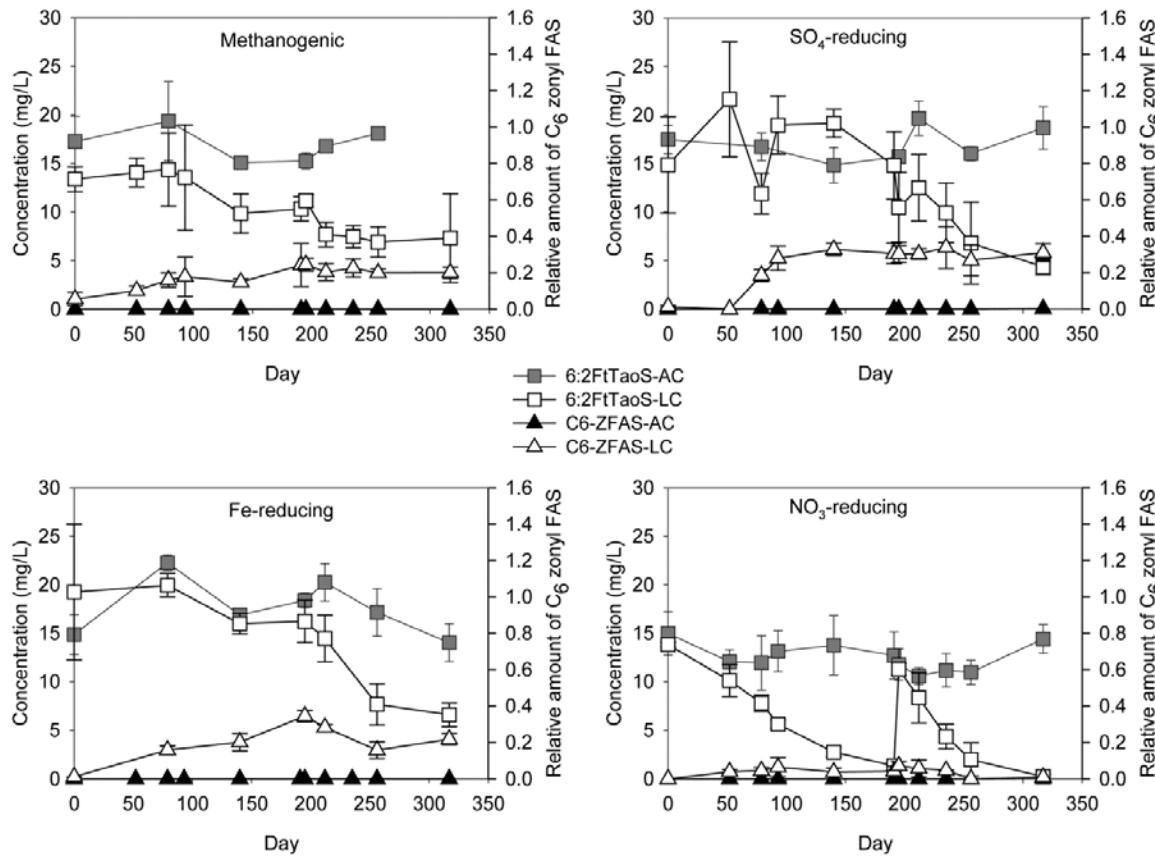
Task 3.1 and 3.2 Anaerobic microcosms with Ansul AFFF

Preliminary results of a 35 d anaerobic incubation with the Ansul AFFF indicate that the soil microbial community can actively use the organic carbon in AFFF under all electron acceptor conditions (data not shown). Visible color changes resulting from the utilization of electron acceptors were apparent in bottles containing live cultures compared to autoclaved controls. Analysis of PFAS concentrations in active and control bottles on days 0, 15 and 35 indicated no significant production of FTSAs or PFCAs under any electron acceptor condition.

Anaerobic biotransformation of the Ansul AFFF formulation was analyzed in a 317-day incubation. PFAS analyses of the microcosms indicated that the 6:2 FTTAoS, the principal fluorotelomer precursor in Ansul AFFF formulation, was gradually transformed under every tested redox condition, compared to the respective autoclaved control microcosms (Fig. 8).

The most active transformation was observed under nitrate-reducing conditions, where the live cultures successfully transformed two consecutive doses of 6:2 FTTAoS in the Ansul AFFF during the incubation period. Compared to the 159 day incubation for the first dose of Ansul AFFF, it took only 61 days for live cultures to transform a similar amount of 6:2 FTTAoS in a second dose of Ansul AFFF, indicating increased microbial transformation of FTTAoS. In contrast, a much slower biotransformation occurred under the other three redox conditions with only about 45-70% removal of the 6:2 FTTAoS in the first dose of Ansul AFFF.

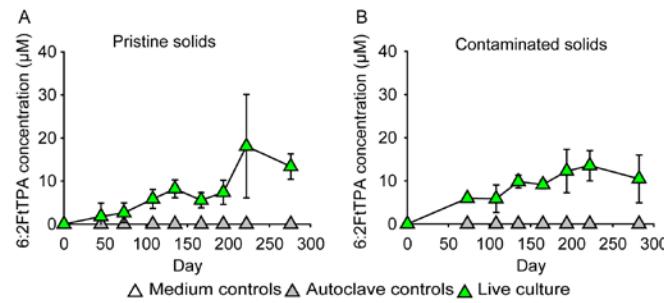
Fig 8. Anaerobic biotransformation of 6:2 FTAAoS in the Ansul AFFF. Note ZFAS is an early acronym for FTTPA.



The TOP assay was performed on all control and live microcosm slurries in order to quantify PFAS mass balances. Compared to the respective autoclaved controls, $121\pm15\%$, $115\pm7\%$ and $96\pm8\%$ of PFASs were recovered on days 0, 194, and 276 from the live microcosms inoculated with pristine solids, and $114\pm10\%$, $98\pm13\%$ and $67\pm6\%$ of PFAS mass was removed on days 0, 135, and 282 with contaminated solids (data not shown). These results suggest that while mass balances were achieved in the pristine solid microcosms, biotransformation in the contaminated microcosms eventually led to non-detectable products.

The identity of 6:2 FTTPA was confirmed by the authentic standard Zonyl FSA. The formation of this intermediate was not observed in the medium or autoclaved controls, but accumulated in the live

Fig 9. Production of 6:2 FTTPA (m/z 451) during 6:2 FtTAoS biotransformation. Error bars represent the standard deviation of averages from triplicate microcosm.



culture (Fig. 9). At the end of the incubation, insignificant difference in the final 6:2 FTTPA concentrations was observed between pristine and contaminated microcosms. The 6:2 FTAAoS was predominantly transformed to 6:2 FTTPA, likely via a hydrolysis reaction that is catalyzed by a microbial amidase. Two additional identified products, fluorotelomer thioether propanoyl alaninate (FTTPIA) and 6:2 fluorotelomer thioether propanoylalanylalaninate (FTTPIAA) were identified (Fig. 10).

Aerobic microcosms with 3M AFFF

Although the experimental procedures were not described in earlier sections, preliminary results of a 20 d aerobic incubation with the

3M AFFF formulation indicated that the soil microbial community can actively use the organic carbon in AFFF to support their growth. Cell counts in the microcosms revealed that initial bacterial numbers in the medium containing only AFFF accounted for 34% of total cells, but this percentage increased to almost 100% in subsequent days, indicating that the organic carbon in AFFF can effectively serve as an energy and carbon source for the microcosm organisms.

Analysis of PFAS concentrations in active and control samples on day 0 and day 20 demonstrated no significant increase in PFCAs and PFSAs, even though 3M AFFF is known to contain C4-C8 precursors.^{2,6} Significant decreases in PFOS were observed both in active and autoclaved soils, suggesting that the PFOS loss is due to abiotic processes, such as sorption to soils, rather than biotransformation. No further experiments were conducted with 3M AFFF for this SERDP project. However, the biotransformation of 3M AFFF will be investigated under SERDP ER-2720, “Key Fate and Transport Processes Impacting the Mass Discharge, Attenuation, and Treatment of Poly- and Perfluoroalkyl Substances and Comingled Chlorinated Solvents or Aromatic Hydrocarbons,” which is being led by Dr. C. Higgins of the Colorado School of Mines.

Aerobic microcosms with Ansul AFFF

Live microcosms amended with Ansul AFFF containing DGBE gave organic carbon concentrations that disappeared within 3 to 5 d, while no similar change was observed for autoclaved/medium controls (Fig. 11). Similar trends in FTAAoS disappearance were observed for 4:2 and 8:2 FTAAoS; however, these forms occurred at lower concentrations in AFFF relative to 6:2 FTAAoS (data not shown).⁴ In live microcosms, oxygen concentrations declined, which indicates that microbial community was active. In contrast,

Fig 10. Proposed anaerobic biotransformation pathway of 6:2 FtTAoS. Red box indicates tentatively-identified products.

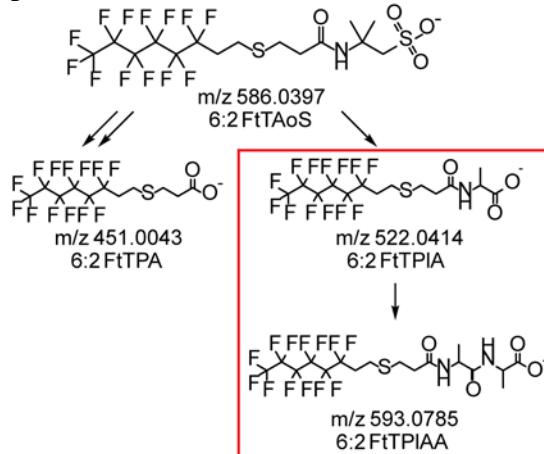
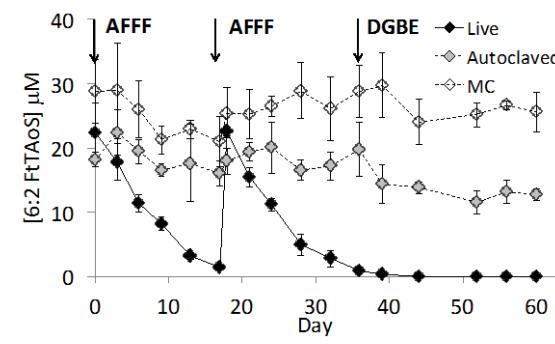


Fig 11. Aerobic biotransformation of 6:2 FTAAoS after multiple additions. Error bars represent the standard deviation of triplicate experimental bottles.⁴



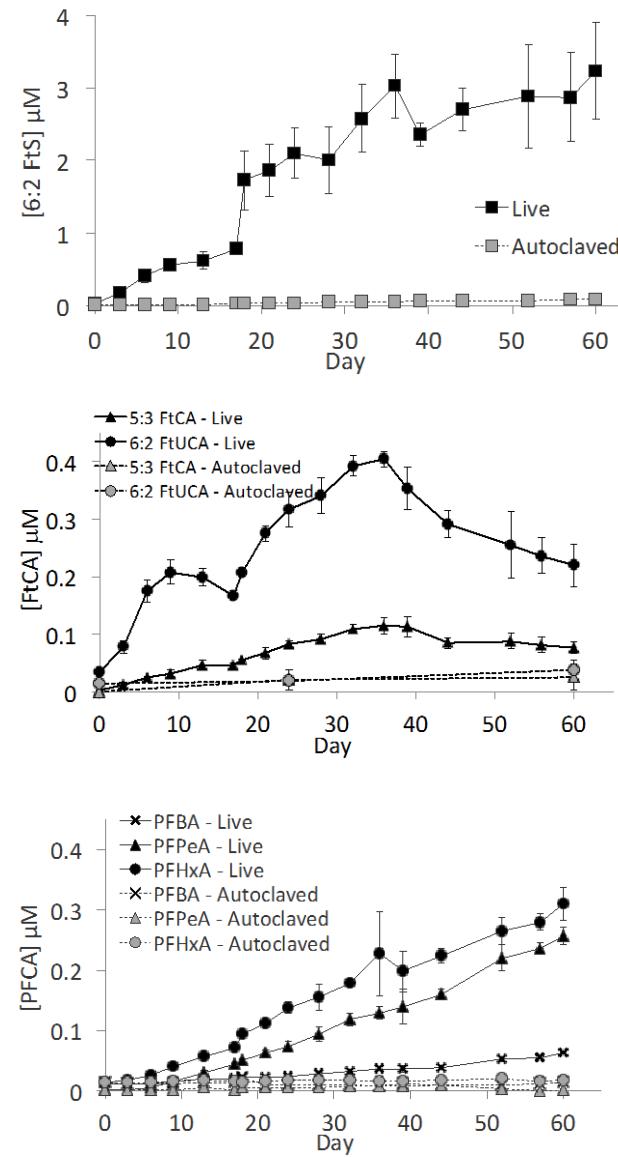
concentrations of FTTAoS remain nearly constant in controls (Fig. 11).

The most abundant transformation product formed was 6:2 FTSA (Fig. 12a), presumably because the 6:2 FTTAoS was the most abundant precursors in the National Foam AFFF,² but only accounted for 8% of the initial mass of 6:2 FTTAoS added. The 4:2 and 8:2 homologs also were detected in the microcosm experiments, but accounted for less than 1% of the corresponding parent FTTAoS (data not shown).⁴ In addition, the 5:3 FTCA and 6:2 FTUCA were formed but only accounted for ~ 0.5% and 0.18% of FTTAoS precursors (Fig 11b). Formation of 8:2 FTUCA was observed but at low concentrations and there were no trends observed for 7:3 FTCA or 6:2 FTCA, relative to autoclaved controls (data not indicated)⁴

Several persistent PFCAs were observed including PFHxA, PFPeA, and PFBA (Fig. 12C) that were 48%, 40%, and 10% of total PFCAs produced, which was ~ 1.5% of the total FTTAoS transformed. It is interesting to note that PFBA is expected to be a product of 4:2 FTTAoS and, by analogy, 4:2 FTSA. However, the concentration of PFBA at the end of the experiments (0.5 μ M) was greater than the amount of 4:2 FTTAoS biotransformed (<0.01 μ M). This finding indicates that PFBA may be a biotransformation product of other chain lengths (e.g., 6:2 and 8:2). However, specific mechanism is not identified. In addition, PFHpA and PFOA were detected. PFCAs were not detected in controls.

An attempt was made to identify additional information products in the live microcosms. Four polyfluorinated QTOF-MS as the sulfone (-SO-) sulfoxide (-SO₂-) and intermediates of the 6:2 and 8:2 FtTAoS. The structures of 6:2 fluorotelomer sulfoxide amido sulfonate (6:2 FTSAOAOs) and 6:2 fluorotelomer sulfone amido sulfonate (6:2 FTSAO₂AoS) are indicated in the overall pathway (Fig 12). Interestingly, the 6:2 sulfone (6:2 FTSAOAOs) was also observed in the original National Foam AFFF as well as in autoclaved

Fig 12. Change in concentration in major transformation intermediates including: 6:2 FTSA (A), 6:2 FTUCA and 5:3 FTCA (B), and PFBA, PFPeA, PFHxA (C).⁴



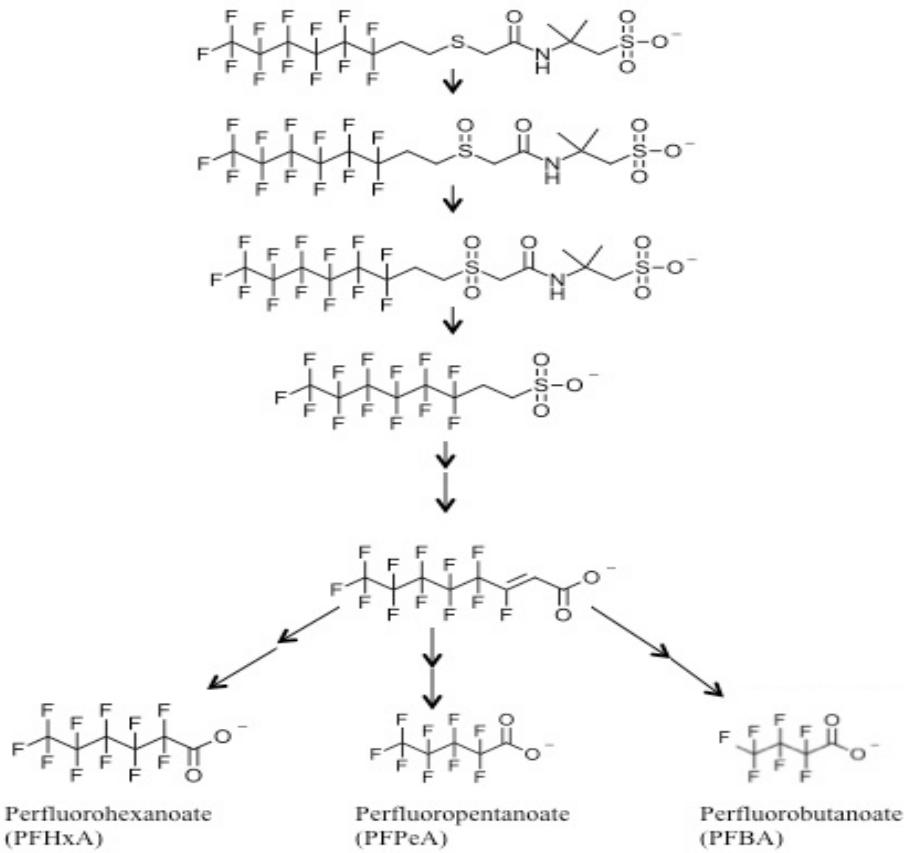
controls but not in medium controls, which suggests an abiotic pathway for the production and loss of the 6:2 sulfone (6:2 FTSAO₂AoS).⁴

Others report the biological oxidation of thioether-containing compounds to sulfones and sulfoxides⁵⁷⁻⁵⁹ and abiotic oxidation the thioether, dimethyl sulfide.⁶⁰ The formation of analogous 4:2 FTSAO₂AoS and 4:2 FTSAO₂AoS were not detected in any microcosms, presumably due to the low concentrations of the 4:2 FTTAoS precursor in National Foam AFFF. This study extends the findings of Weiner et al.,⁵⁴ who proposed the sulfone and sulfoxide products. However, they were unable to ascertain whether the sulfone was a product of biotransformation because it was observed in both live and control microcosms and no 6:2 FTSAO₂AoS (sulfone) was detected in their study.

Mass Balance. All the transformation products observed accounted for an estimated 10% of the transformed FTTAoS. Please note that accurate measurements of the sulfone and sulfoxide intermediates are confounded by the lack of standards. Mass balance calculations are also challenging because only an authentic reference material was available for this 6:2 FTTAoS precursor, and not for the 4:2 and 8:2 analogs. Further, stable-isotope labeled standards were not available for any of the FTTAoS precursors. Because mass balance was not obtained, the TOP assay was applied to microcosm samples. The TOP assay recovered 75-85% of the added FTTAoS in the form of PFCAs and up to 80-100% during the second AFFF additions.⁴ Additional research would be required to identify the remaining transformation products that are detected by the TOP assay. Application of the TOP assay to the microcosm study, demonstrates its potential utility for closing the mass balance, although it cannot be used to identify additional transformation products.

Environmental Implications. The initial biotransformation of FTTAoS occurs in a matter of weeks under aerobic conditions, but subsequent conversion to PFCAs is slow, even under aerobic conditions. The identification of sulfones, sulfoxides, and FTSAAs in microcosms is important, because it rationalizes their occurrence in anoxic AFFF-contaminated groundwater.⁴¹ Although FTSAAs undergo anaerobic transformation to FTCAs and PFCAs,¹² they do not biotransform under anaerobic conditions.¹³ The lack of anaerobic FTSA biotransformation is consistent with the high (ug/L to mg/L) levels of FTSAAs found in anoxic AFFF-contaminated groundwater.^{2,10} Based on the proposed pathway (Fig 13), FTSAAs in an anoxic groundwater that reach aerobic receiving waters, will potentially undergo biotransformation to dead-end PFCAs, thus acting as a long-term source of PFCAs to surface waters.

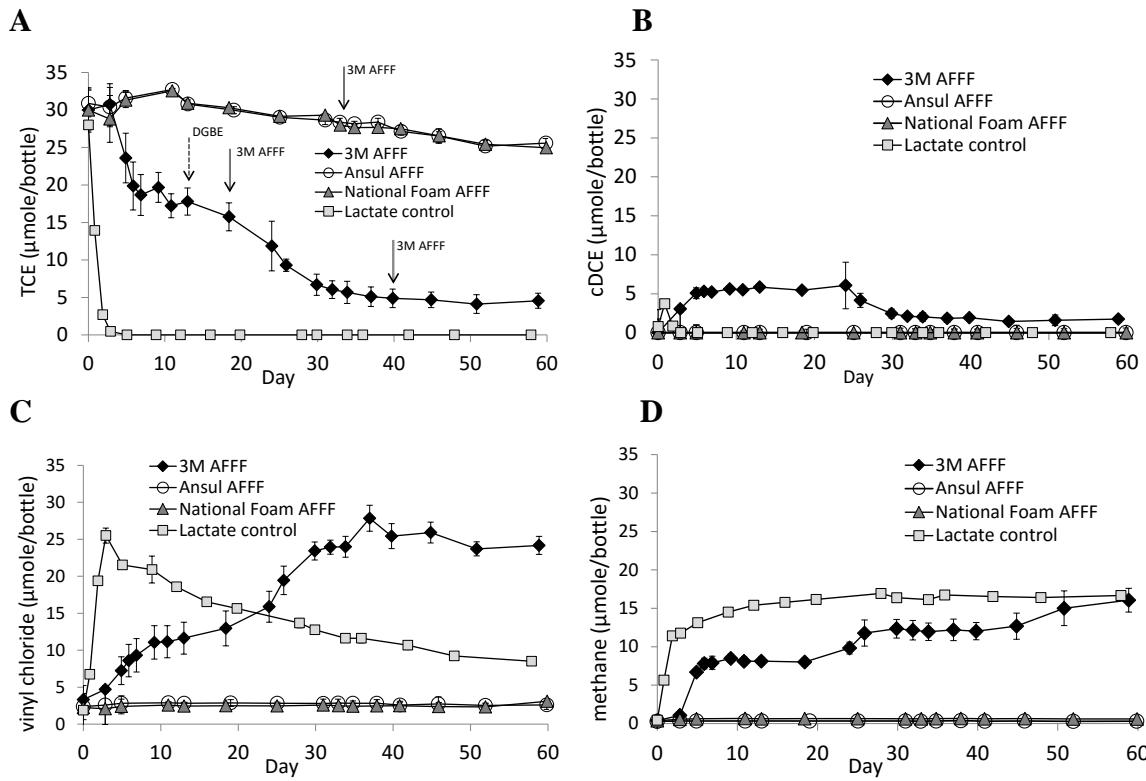
Fig 13. Proposed biotransformation pathways of 6:2 FTTAoS.⁴



Task 3.3 Impact of AFFF on TCE biotransformation

AFFF Amendment Experiments. Reductive dechlorination was observed in microcosms containing 3M AFFF, but not in those amended with either Ansul or National Foam AFFF (Fig 14 A-C). Subsequent amendments of DGBE and 3M AFFF (Fig 14A) indicated that hydrogen limitation after DGBE amendment was responsible for the slow dechlorination rate and lack of methanogenic activity (Fig 14D). Low concentrations of hydrogen and slow rates of dechlorination after DGBE amendment compared to that after addition of 3M AFFF indicates that other fermentable substances are present in 3M AFFF, which form acetate and hydrogen. However, the addition of 3M AFFF to the systems containing National Foam and Ansul did not result in increased rates of TCE dechlorination. Autoclaved controls and growth medium only controls did not produce significant amounts of methane and TCE dechlorination did not occur.

Fig 14. TCE (A) cis-dichloroethene (DCE) (B), vinyl chloride (C), and methane (D) concentrations in microcosms containing AFFF (3M, National Foam, and Ansul) or just lactate. Amendment additions indicated by arrows.⁵



Although acetate was produced by cultures receiving National Foam AFFF, it did not result in TCE reductive dechlorination. Increased acetate production in the National Foam amended systems is attributed to ethylene glycol, which is transformed to acetate under anaerobic conditions.^{61,62} No significant growth of *D. mccartyi* growth as detected, as measured by 16S rRNA gene copy numbers. None of the PFAS concentrations declined significantly for any of the test systems as well as the controls.

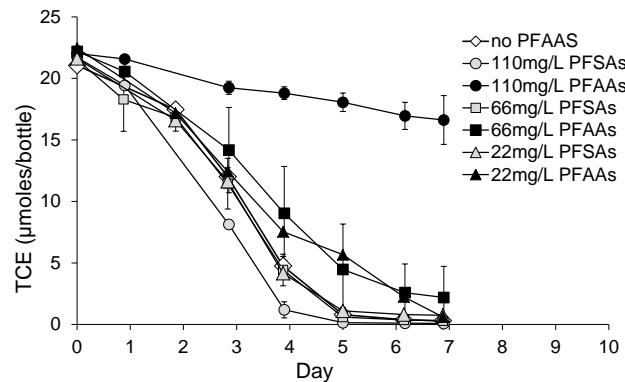
DGBE Amendment Experiments. Experiments were performed to determine if DGBE could act as an electron donor upon fermentation to drive TCE reductive dechlorination. Although cultures were provided enough substrate (lactate or DGBE) to have an excess of electron equivalents to dechlorinate TCE, there was not a significant difference between cultures receiving lactate and DGBE from those amended with only lactate (Fig 14). This finding indicates that lactate is more labile for fermentation than DGBE.

AFFF Component and PFAS Amendment Experiments. To gain further insight into the inhibitory effect of National Foam and Ansul AFFFs on TCE dechlorination, individual components of the AFFF were added to culture undergoing lactate- enhanced fermentation. Reductive dechlorination of TCE was not inhibited by the addition of 18 mg/L ethylene glycol, 12 mg/L 1-propanol, or 45 mg/L 6:2 FTTAoS, the main active component in Ansul AFFF. In contrast, the addition of 16 mg/L zwitterionic 6:2 FTSaB slowed the rate of reductive and doubling the concentration of 6:2 FTSaB to 32 mg/L inhibited reductive dechlorination

altogether (data not shown). National Foam AFFF contains zwitterionic betaines and cationic surfactants. Non-fluorinated zwitterionic and cationic surfactants are established anti-microbial agents.⁶³⁻⁶⁵

The addition of a mixture of PFSAs and PFCAs added at 22 and 66 mg/L did not significantly impact the rates of TCE dechlorination (Fig. 15). In contrast, a mixture of PFSAs and PFCAs (100 mg/L) significantly reduced the rate of reductive dechlorination (Fig 15). Given this observation, either or the combination of PFCAs and PFSAs or PFCAs alone cause of inhibition of TCE dechlorination. Weathers et al. 2016 found that the abundance of *D. mccartyi* decreased when exposed to a mixture of PFSAs and PFCAs (110 mg/L), when compared to controls that contained no PFSAs and PFCAs.⁴⁷

Fig 15. Impact of additional PFSAs or a mixture of PFSAs and PFCAs on the reductive dechlorination of TCE.⁵



Environmental Implications. Based on the experiments with AFFF and TCE, it appears that the ability to support reductive dechlorination of TCE depends on the composition of the AFFF. Fermentation of the organic solvents, such as DGBE and ethylene glycol, drives the production of hydrogen and acetate, which are necessary to support TCE dechlorination by *D. mccartyi*. In the case of DGBE, the rates of reductive dechlorination are lower due to the lower concentrations of hydrogen produced when compared to other potential constituents, such as ethylene glycol. PFCAs may impact reductive dechlorination of TCE. Ongoing transformation of precursors, due to biotransformation or oxidation, to persistent PFCAs may potentially impact the activity of microbial communities carrying out reductive dechlorination.¹⁶ Because a range of AFFFs was likely used at sites,^{2,10,16} the actual impact of AFFF addition of an individual site is difficult to predict.

Task 4 Sorption of Cationic and Zwitterionic PFASs to Soils and Sediments

Sorption Isotherms. Plots of the aqueous phase concentration (C_w) against the soil phase concentration (C_s) were nonlinear for most soils and analytes, as indicated by $n \neq 1$ in the linearized Freundlich isotherms (Table 6). The values of n typically ranged from 0.8 to 1.2, which is consistent with other batch sorption experiments for PFCAs and PFSAs.^{22,23,49}

Capstone Product vs. AFFF. Preliminary experiments compared the sorption of the 6:2 FTsAb from Capstone, a commercial mixture that contains 6:2 FTsAb, 6:2 FTS, and 6:2 FtSaAm but is not an AFFF, and from the National Foam AFFF demonstrated that the sorption of the 6:2 FTsAb was not significantly different at the 95% confidence level (CI). Therefore, multiple competing sorption processes (e.g. van der Waals, electrostatics) driving the sorption of 6:2 FTsAb in the presence of hydrocarbon surfactants and additional solvents in the AFFF result in no difference in sorption of the 6:2 FTsAb. Additional analyte comparisons between the commercial product and the AFFF could not be made, since the remaining two analytes in the

commercial product were either not removed (6:2 FTSA) or completely removed (6:2 FTSaAm) from the aqueous phase. Similar phenomena were observed with sorption of PFCAs and PFSAs in the presence of an anionic hydrocarbon surfactant.²²

Table 6. Freundlich coefficients ($\log K_f$), n values, and correlation coefficients (R^2) for the 6:2 FTSA, 8:2 FTSA, 6:2 FTSaB, 8:2 FTSaB, 10:2 FTSaB, and 6:2 FTSaAm Freundlich isotherms for Soils 1-6, Soil 1 pH 4, and Soil 1 pH 7.³

Soil #	Fit Parameters	6:2 FTSA	8:2 FTSA	6:2 FTSaB	8:2 FTSaB	10:2 FTSaB ^a	6:2 FTSaAm
1	$\log K_f$	0.67 ± 0.25^b	1.7 ± 0.090	1.4 ± 0.23	2.2 ± 0.15	2.3 ± 0.22	n/a ^c
	n	1.1 ± 0.098	1.1 ± 0.11	1.2 ± 0.093	1.3 ± 0.11	1.2 ± 0.18	n/a
2	$\log K_f$	0.86 ± 0.11	1.28 ± 0.30	2.1 ± 0.22	2.3 ± 0.26	2.0 ± 0.14	n/a
	n	0.74 ± 0.084	0.83 ± 0.32	0.91 ± 0.23	0.49 ± 0.52	0.59 ± 0.39	n/a
3	$\log K_f$	0.87 ± 0.10	1.5 ± 0.16	0.91 ± 0.38	1.7 ± 0.41	n/a	n/a
	n	0.82 ± 0.078	0.97 ± 0.18	1.3 ± 0.25	1.7 ± 0.68	n/a	n/a
4	$\log K_f$	1.1 ± 0.070	2.3 ± 0.40	0.63 ± 0.72	1.7 ± 0.39	n/a	n/a
	n	0.98 ± 0.060	1.2 ± 0.36	1.5 ± 0.45	1.8 ± 1.1	n/a	n/a
5	$\log K_f$	0.83 ± 0.18	1.6 ± 0.15	1.8 ± 0.24	n/a	n/a	n/a
	n	0.88 ± 0.13	1.1 ± 0.18	0.90 ± 0.18	n/a	n/a	n/a
6	$\log K_f$	0.84 ± 0.070	1.6 ± 0.12	1.9 ± 0.21	2.4 ± 0.13	n/a	2.2 ± 0.20
	n	0.95 ± 0.058	1.1 ± 0.13	0.91 ± 0.17	0.41 ± 0.34	n/a	0.17 ± 0.23
1 pH 4	$\log K_f$	0.83 ± 0.15	1.7 ± 0.19	2.0 ± 0.22	2.3 ± 0.12	2.7 ± 0.19	n/a
	n	0.93 ± 0.12	0.90 ± 0.20	0.80 ± 0.16	0.85 ± 0.21	0.96 ± 0.37	n/a
1 pH 7	$\log K_f$	0.78 ± 0.17	1.2 ± 0.14	1.4 ± 0.17	2.1 ± 0.14	n/a	n/a
	n	0.85 ± 0.13	0.97 ± 0.19	0.97 ± 0.11	0.83 ± 0.23	n/a	n/a

^aThe 12:2 FTSaB is not included since all aqueous phase concentrations were below the limit of detection. ^bError represents the 95% CI of the y-intercept ($\log K_f$) or slope (n). ^cNot applicable.

Soil 1 Native pH (5.1) Isotherm. Linearized Freundlich isotherms (Eqn 2; Fig. 15) of the anionic 6:2 and 8:2 FTSA and the zwitterionic 6:2, 8:2, and 10:2 FTSaB for Soil 1 at pH 5.1 indicates the relative sorption strength of each sorbate. The values of the y-intercepts (i.e. $\log K_f$) indicate that the strongest sorbed analyte is the 10:2 FTSaB, followed by the 8:2 FTSaB, 8:2 FTSA, 6:2 FTSaB, and 6:2 FTSA, which is the weakest sorbed analyte. Within each class (FTSA and FTSaB), sorption increases as the fluorinated chain length increases. The increase in sorption with fluorinated chain length for PFCAs and PFSAs was observed in previous sorption studies.^{23,27,33} The vertical increase of the last 3 points in the 6:2 FTSA and 6:2 FTSaB isotherms strongly suggests that sorption is no longer following monolayer sorption but becomes multilayer sorption at the highest sorbate concentrations.⁶⁶ The cationic 6:2 FTSaAm and the zwitterionic 12:2 FTSaB were not detected (< LOD) in the aqueous phase and were completely sorbed to the soil. For the purposes of brevity, only the isotherms for Soil 1 at the native pH are discussed here; isotherms for all analytes for Soils 2-6, and for Soil 1 pH 4 and pH 7 are not shown.

FTSA, FTSaB, and 6:2 FTSaAm

Sorption. The $\log K_d$ increase per CF_2 group between the 6:2 and 8:2 homologs for the FTSA and FTSaBs was 0.41 ± 0.059 and 0.20 ± 0.088 , respectively. The increase in $\log K_d$ per CF_2 group for the anionic FTSA is consistent with the 0.5-1 $\log K_d$ increases for the anionic PFCAs and PFSAs.^{23,26,67} The $\log K_d$ increase per CF_2 group for the zwitterionic FTSaBs is lower than expected $\log K_d$ increase for the anionic FTSA. However, the analytical variability was greater for the FTSaBs, due to a lack of internal standards, relative to the FTSA.

For the same fluorinated chain length, the 6:2 FTSaB has a larger K_d than the 6:2 FTSA for all soils and pH

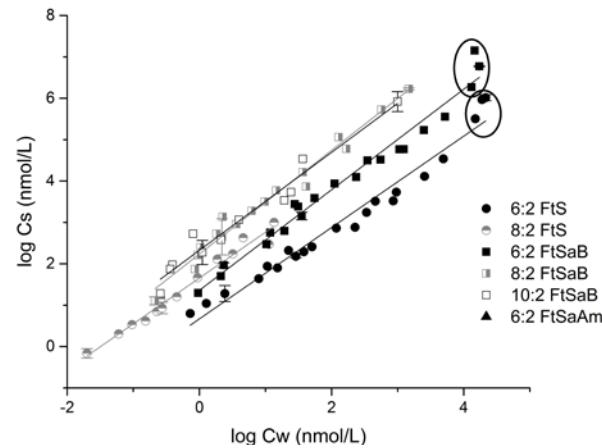
conditions, despite the greater size and molecular weight of the 6:2 FTSaB. Since the 6:2 FTSaB head group has two positive charges and a terminal negative charge at pH 5.1 (Fig. 2), the head group is contributing to increased interactions, presumably due to cation exchange. The 6:2 FTSaAm, which was completely sorbed to all soils except Soil 6, has two positive charges (Fig. 2), one of which is terminal. The 6:2 FTSaAm has the highest observed K_d value (Table A8), so the number of positive charges cannot explain the difference in sorption. Rather, the position of the charges is important; in this case, the terminal positive charge on the 6:2 FTSaAm leads to greater sorption onto soil.

The single-point K_d values for the 6:2 FTSA (3.1-12 L/kg; Table A8) are in good agreement with the field- K_d values reported (5.8 L/kg²⁹ and 3.2 L/kg³²) for sediment-water systems. The K_d values for the 6:2 FTSaB range from 23-240 L/kg (Table A8), which are significantly greater than the single field- K_d of 4.5 L/kg.²⁹ The single K_d value obtained for the cationic 6:2 FTSaAm was 650 L/kg (Table A8), which is also significantly greater than the single reported field- K_d of 34 L/kg.³² The lower values reported by Boiteux et al.²⁹ are likely due to incomplete extraction of zwitterionic and cationic PFASs from sediment using the milder extraction conditions (1% acetic acid in methanol) compared to the 0.5 M HCl in methanol that was used in the present study.

Correlations. Sorption of anionic FTSA does not indicate a trend in K_d with organic carbon (Fig. 16). The K_d values for the longer-chained 8:2 FTSA are consistently greater than those of 6:2 FTSA except for Soil 4 ($K_d = 120$ L/kg), in which the 8:2 FTSA K_d is disproportionately high for an unknown reason. At the lowest levels of organic carbon (0.098 – 0.12%), the K_d values are similar in magnitude to the soils with higher organic carbon (1.0 – 2.3%), which suggests that the mineral phase in the low organic carbon soils (Soils 2, 6) influences sorption.^{24,25,32} The nonpolar fluorinated tail of the anionic FTSA may interact with the hydrophobic (nonpolar) sites between the charged sites on the mineral surface⁶⁸ via weak hydrophobic interactions.^{24,69}

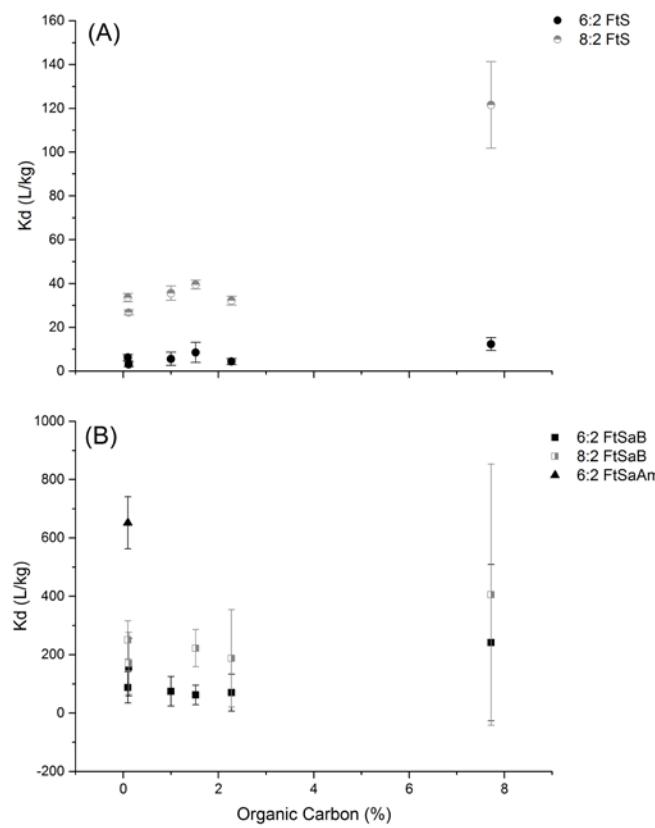
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Fig. 16. Freundlich isotherms (log-log scale) for anionic 6:2 and 8:2 FTSA and the zwitterionic 6:2, 8:2, and 10:2 FTSaBs (Soil 1). The ovals highlight the significant increase in sorption at the highest concentrations.³



The lack of a correlation in FTSA sorption may be due to the extent of organic matter decomposition of the soils tested. If the C/N ratio is accepted as a rough proxy for the extent of organic matter decomposition, with a wide C/N indicating a relative prevalence of aliphatic C and nonpolar functionality, the quality of organic matter can be determined.⁷⁰ Soils 1-3 and 5 have either low organic carbon content or a lower C/N ratio (greater number of carboxyl groups; Table 1), indicating a minimal influence of organic carbon on sorption (Soil 2) or less hydrophobic organic matter (Soils 1, 3, and 5). Therefore, the lack of correlation of FTSA sorption with soil organic carbon may be due to low hydrophobicity of the organic matter of the soils tested, with the exception of Soil 4, which is more hydrophobic (higher C/N ratio; Table 1) with fewer carboxyl groups.

Fig. 17. No correlation between (A) the anionic FTSA_s and (B) the zwitterionic FTSA_sBs and cationic 6:2 FTSA_sAm and soil organic carbon.³



To the best of our knowledge, studies examining PFAS sorption do not consider metrics for the extent to which decaying organic matter that has ionizable, oxygen containing functional groups, which needs to be examined further. Many sorption studies of PFCAs and PFSAs observe an increase in K_d with increasing organic carbon,^{23,24,49,71} while a single study found little change in K_d with increasing organic carbon.⁷²

The sorption of zwitterionic 6:2 and 8:2 FTSA_sBs also do not correlate with organic carbon (Fig. 17), which is consistent with reports that K_d values for zwitterionic pharmaceuticals do not correlate with organic carbon content.^{36,37} The K_d values for the 8:2 FTSA_sB are consistently and proportionally higher than the 6:2 FTSA_sB, due to the increased hydrophobicity of the additional two CF₂ groups. Due to the proportionate increase in K_d for the FTSA_sBs with organic carbon, the disproportionate increase in the 8:2 FTSA K_d in Soil 4 (Fig. 17a) may be an outlier.

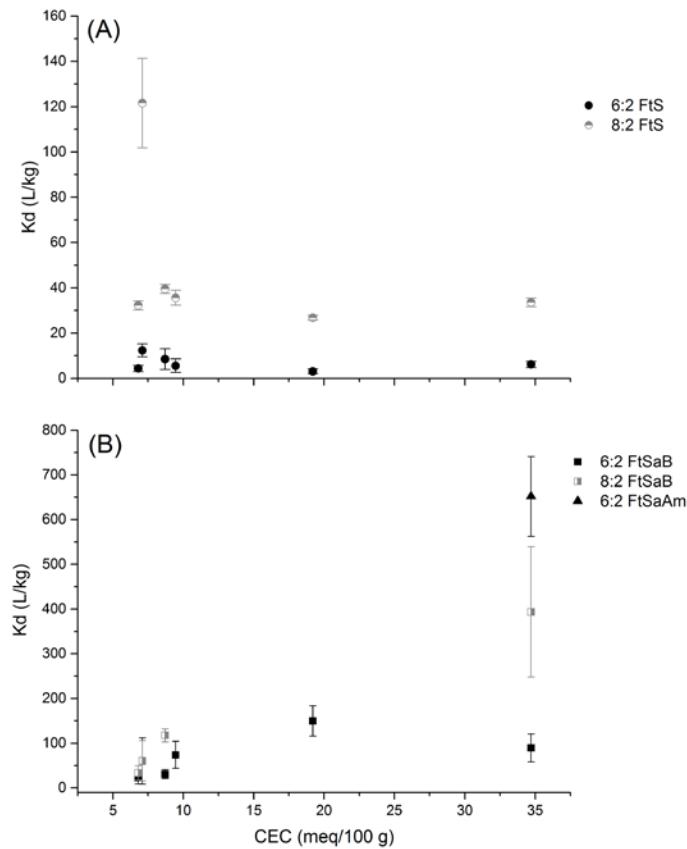
Interestingly, the magnitude of the K_d values for the zwitterionic FTSA_sBs are higher than the anionic FTSA_s, which is likely due to the polar head group, as will be discussed below.

Not surprisingly, the sorption of the anionic 6:2 and 8:2 FTSA_s does not correlate with CEC (Fig. 18a), due to repulsion between negatively-charged organic matter and mineral surfaces and the negatively charged sulfonate group. The high K_d of 120 L/kg for the 8:2 FTSA in Soil 4 with a CEC of 7.1 meq/100 g is attributed to the more hydrophobic nature of the organic matter in this

soil as discussed above rather than cation exchange processes.

Sorption of the zwitterionic 6:2 FTsAsB does not correlate with increasing CEC, while the K_d values for the 8:2 FTsAsB may increase with increasing CEC (Fig. 18b). The proportionate increase in K_d from the 6:2 FTsAsB to the 8:2 FTsAsB may arise from the greater affinity of the 8:2 FTsAsB to the hydrophobic sites in between the localized negative charges on the smectite surface.⁶⁸ Interestingly, the cationic 6:2 FTsAsAm was completely depleted from the aqueous phase in all but one soil (Soil 6), which has the highest CEC (35 meq/100 g). The terminal positive charge in the cationic 6:2 FTsAsAm may interact more readily with the cation exchange sites than the FTsAsBs, in which the terminal carboxyl group of the FTsAsBs may repel the negatively-charged CEC sites.⁷³ Regardless, sorption studies with zwitterionic and cationic veterinary pharmaceuticals^{36,37} and cationic hydrocarbon surfactants⁷⁴ report that CEC is the major sorption driver.

Fig. 18. No correlation between (A) the anionic FTsAs and cation exchange capacity and a potential positive correlation for the zwitterionic 6:2 and 8:2 FTsAsBs (B) with CEC.³



The negative charge (cation exchange sites) of sorbent surfaces in soils likely arises from mineral as well as organic matter constituents.⁷⁵ Soil organic matter develops negative charges as the oxidative decomposition process acting on plant litter adds carboxyl groups with pK_{as} in the range of 4-6.⁷⁶ Phyllosilicates develop permanent negative charge through isomorphous substitutions within Al-octahedra or Si-tetrahedra of the mineral phase.^{68,77} The extent of negative charge is conveniently expressed by the capacity of the soil matrix to adsorb and exchange cations with the soil solution (CEC). Soil 6 is unique with regards to CEC, since the clay fraction of Soil 6 is dominated by smectite with low (0.098%) organic carbon. Soil 2, which also has low organic carbon and a high CEC (but half that of Soil 6), contains some smectite as well as pedogenic iron oxides and other clay minerals. Although Soils 2 and 6 have similar organic carbon and CEC, the additional minerals and pedogenic iron oxides present in Soil 2 may provide additional mechanisms necessary to sorb the cationic 6:2 FTsAsAm, since sorption of some organic cations is influenced by both CEC and organic carbon.⁷⁵ The greater complexity of the mineral phase in Soils 1-5 indicates the possibility of sorbate-sorbent interactions through a variety of mechanisms not limited solely to cation exchange, although the exact mechanisms need to be determined.

The lack of a correlation between the zwitterionic 6:2 FTSaB and CEC and the single K_d value for the cationic 6:2 FTSaAm for Soil 6 (highest CEC) suggests that the bulk soil parameters do not fully explain the sorption mechanisms of zwitterions and cations. The easily-measured bulk soil parameters indicate the total number of charged sites, rather than the actual accessibility (spatial and steric effects) of zwitterions and cations to the charged sites (i.e. sites for CEC, cation bridging, anion ligand exchange).⁷⁸

The K_d model proposed by MacKay and Vasudevan⁷⁸ incorporates the accessibility of charged sites:

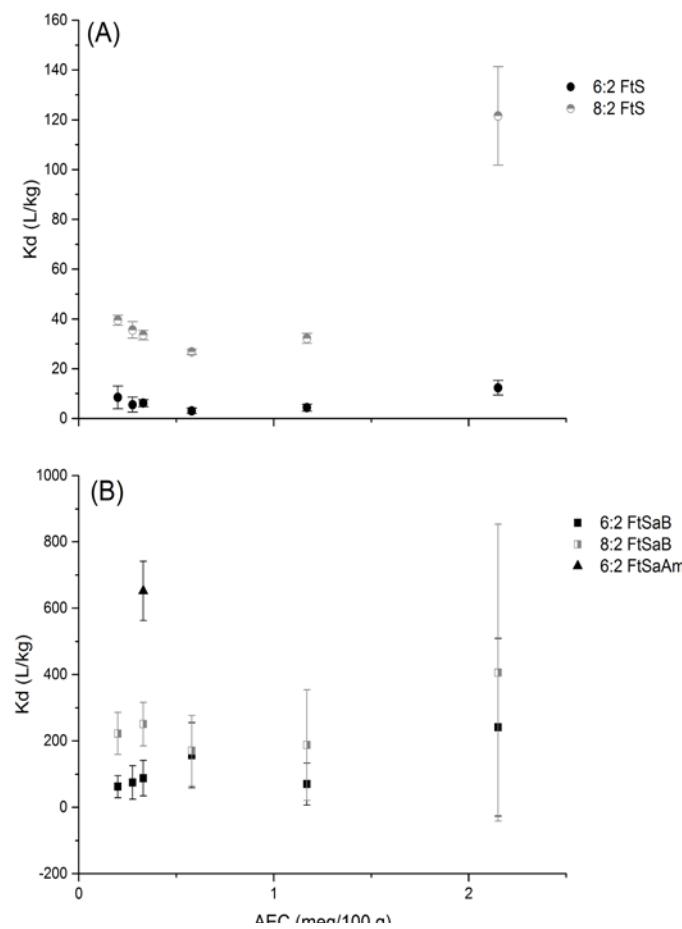
$$K_d = \frac{C_{s,Type\ I} + C_{s,CE} + C_{s,CB+LE}}{C_w} \quad (\text{Eqn 3})$$

where $C_{s,Type\ I}$ is the analyte concentration on the soil due to hydrophobic interactions. The K_d model of MacKay and Vasudevan⁷⁸ indicates that a combination of hydrophobic interactions (i.e. hydrophobic sites or more hydrophobic organic matter) and exchange processes contribute to the overall sorption of zwitterions and cations, which is likely the case with the zwitterionic FTSaBs and the cationic 6:2 FTSaAm.

Surprisingly, sorption of the anionic FTSA remains unchanged as AEC increases (Fig. 19a), with the exception of the high K_d value of the 8:2 FTSA for Soil 4 (AEC = 2.2 meq/100 g). The sorption of the 8:2 FTSA is proportionally greater than the 6:2 FTSA, except in Soil 4. Since Soil 4 has both the highest organic carbon content and the highest AEC, the exact sorption mechanism is difficult to determine and may be a combination of the two parameters.

Anion exchange sites are fully protonated ($\text{pH} \leq 6$), singly coordinated hydroxyl groups that arise from poorly crystalline forms of iron oxides (ferrihydrite) and aluminosilicates.⁷⁹ Soil 3 is a particularly oxide rich Ultisol, and Soil 4 has a poorly crystalline mineral phase (“andic” soil properties) that contains singly coordinated hydroxyl groups. In the case of Soil 4 with the anionic 8:2 FTSA, inner sphere complex formation via anionic ligand exchange with the singly coordinated hydroxyl groups⁷⁹ may contribute to the observed increase in sorption. However, if

Fig 19. Lack of correlations for the (A) anionic FTSA and (B) zwitterionic FTSaBs and cationic 6:2 FTSaAm as a function of AEC. Error bars indicate the 95% CI.³



inner sphere complex formation was driving sorption in Soil 4, a proportional increase in the sorption of the 6:2 FTSA would be expected. The lack of correlation between FTSA sorption and AEC is in direct contrast to anionic pesticide sorption experiments, which found an increase in K_d with increasing AEC.^{37,79}

The sorption of the zwitterionic FTSaBs does not change with AEC (Fig. 19b). Similar to previous bulk soil parameters, the 8:2 FTSaB is more strongly sorbed than the 6:2 FTSaB. The magnitude of the K_d values of the zwitterionic FTSaBs are greater than the K_d values for the anionic FTSA. AEC trends with zwitterion sorption are less frequently studied, but zwitterionic pesticide sorption experiments found either an increase⁸⁰ or no change⁸¹ in sorption with increasing AEC. The differing conclusions suggest that the location of the charge may influence zwitterion sorption by anion exchange processes. Although the FTSaBs have a terminal carboxyl group, the two positively charged moieties appear to have a larger impact on sorption.

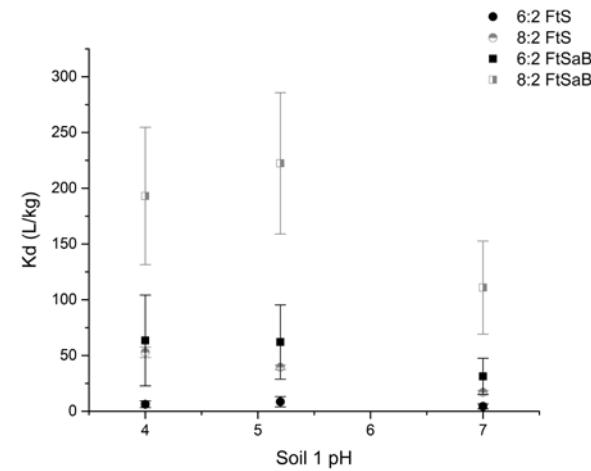
As pH increases from 4 to 7, K_d values remain unchanged for the anionic 6:2 FTSA but decrease for the anionic 8:2 FTSA (Fig. 20). For the zwitterionic FTSaBs, however, sorption remains unchanged as pH increases (Fig. 20).

The decrease in K_d with increasing pH for the anionic 8:2 FTSA is consistent with observed decreases in sorption for organic acids⁸² and other anionic PFASs²³ with an increase in pH. The decrease is linear, with a decrease of 12 L/kg in the K_d with a unit increase in pH (-0.17 log units per unit increase in pH), which is less steep relative to other anionic PFASs.²³ Since the 6:2 and 8:2 FTSA remain anions in the pH range studied, the decrease in sorption is likely due to deprotonation of the negatively charged organic matter ($pK_a \sim 4.5$) and thus anion repulsion.

Alternatively, the singly coordinated hydroxyl groups of the iron and aluminum oxides that contribute to AEC may not be fully protonated ($pK_a \sim 8.5$),⁷⁹ and anionic ligand exchange may not occur as frequently at pH 7, which would also result in the lowest K_d .

The lack of correlation of the zwitterionic FTSaBs with increasing pH may be due to the speciation of the FTSaBs. At pH 4, the FTSaBs have 2 positive charges and ~97% deprotonated carboxyl group (negative charge). At pH 7, the FTSaBs have a negative carboxyl group, a positively charged quaternary amine, and ~95% protonated sulfonamide N. Therefore, the speciation of the zwitterionic FTSaBs does not significantly change in the pH range studied. The minimal change in K_d values suggest that the deprotonation of the negatively charged organic matter has little influence on the sorption of the FTSaBs. The finding contrasts sorption studies of veterinary pharmaceuticals that found pH to play a significant role in zwitterion sorption.^{36,37,81,83}

Fig 20. K_d vs. pH for the anionic 6:2 and 8:2 FTSA and the zwitterionic 6:2 and 8:2 FTSaBs (Soil 1).³



DELIVERABLES

- Improved understanding of the PFAS components of AFFF formulations stockpiled and potentially released to the environment
- New analytical methodology for a comprehensive list of PFASs that occur in groundwater and soil
- New methodology that quantifies groundwater and sediment mixtures' potential to form dead-end PFASs
- Improved understanding of the scope and scale of military site contamination by AFFF components
- New information on the biotransformation of precursors in Ansul AFFF that biodegrade to FTSAs, FTCAs, and PFCAs
- New information on AFFF influence on transport of priority pollutants and transformation of TCE
- Interactions with Higgins' project (ER-2126) for additional leverage and understanding of transport processes
- 8 peer-reviewed articles in Environmental Science and Technology and 1 paper in Environmental Science and Technology Letters
- 5 Ph.D. graduate students and 1 undergraduate received their degrees working on this project

APPENDIX

Table A7. Concentrations of the PFASs quantified in archived groundwater samples from Wurtsmith AFB (WAFB), Naval Air Station Fallon (NASF), and Tyndall AFB (TAFB). Legacy PFAS concentrations are reported in Schultz et al.¹⁰

	TAFB TY22FtA	TAFB T 11-2	TAFB PW-7	TAFB PW-10	NASF MW 16	NASF MW 51-U	WAFB FT-3
Analyte	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
4:2 FTTAoS ^b	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6:2 FTTAoS	<LOD	<LOD	<LOD	8.8	<LOD	<LOD	<LOD
PFBSaAm ^c	4.1 ^c	11	<LOD	720	<LOD	550	26
PFPeSaAm ^c	2.8 ^c	7.8	5.1	190	<LOD	61	79
PFHxSaAm ^c	5.7	8.3	6.3	260	<LOD	260	36
PFBSaAmA ^c	<LOD	<LOD	62	660	<LOD	9.7	<LOD
PFPeSaAmA ^c	<LOD	<LOD	7.9	610	<LOD	5.8	<2.7
PFHxSaAmA ^c	<LOD	<LOD	10	590	<LOD	38	<2.7

^ano 8:2 FTTAoS, 6:2 FTTHN+, fluorotelomer sulfonamido betaines (FTSaB), fluorotelomer sulfonamido amines (FTSaAm);^bcalculated assuming equal molar response to 6:2 FTTAoS;^c calculated assuming equal molar response to PFOS; <LOQ = below the lowest level of quantification

Table A8. Computed K_d values^a and the corresponding C_w (nmol/L) for the 6:2 FTSA, 8:2 FTSA, 6:2 FTSaB, 8:2 FTSaB, 10:2 FTSaB,^b and 6:2 FTSaAm using the 5,000 ng/L C_w and the 60,000 ng/L C_w .³

Soil #		6:2 FTSA	8:2 FTSA	6:2 FTSaB	8:2 FTSaB	10:2 FTSaB ^a	6:2 FTSaAm
1	C_w in 5,000 ng/L C_w R ^c	2.4	<LOQ	2.1	<LOQ	<LOQ	<LOD ^e
	C_w in 5,000 ng/L C_w R2	2.6	0.022	2.4	0.21	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R3	2.4	0.019	2.5	0.22	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R4	2.4	0.019	2.4	0.21	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R1 ^c	29	0.27	36	1.8	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R2	29	0.28	35	2.7	1.2	<LOD
	C_w in 60,000 ng/L C_w R3	29	0.27	35	2.1	1.0	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	5.9 ± 2.0	30 ± 4.2	31 ± 10	120 ± 15	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	8.5 ± 4.6	39 ± 2.0	62 ± 33	220 ± 63	240 ± 83	n/a
2	C_w in 5,000 ng/L C_w R1	3.0	<LOQ	0.71	<LOD	<LOD	<LOD
	C_w in 5,000 ng/L C_w R2	3.1	0.028	0.69	<LOQ	<LOD	<LOD
	C_w in 5,000 ng/L C_w R3	3.0	0.021	0.76	<LOD	<LOD	<LOD
	C_w in 5,000 ng/L C_w R4	3.0	0.020	0.75	<LOQ	<LOD	<LOD
	C_w in 60,000 ng/L C_w R1	42	0.11	11	2.4	1.5	<LOD
	C_w in 60,000 ng/L C_w R2	42	0.15	11	2.4	1.4	<LOD
	C_w in 60,000 ng/L C_w R3	42	0.14	12	2.3	1.4	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	5.7 ± 1.1	39 ± 14	150 ± 34	n/a	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	3.1 ± 1.1	27 ± 1.1	160 ± 98	170 ± 110	93 ± 28	n/a
3	C_w in 5,000 ng/L C_w R1	2.9	<LOQ	5.0	0.34	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R2	3.6	0.024	7.4	0.72	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R3	3.6	<LOQ	4.3	<LOQ	<LOD	<LOD
	C_w in 5,000 ng/L C_w R4	3.8	0.024	7.5	0.63	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R1	32	0.16	56	1.9	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R2	32	0.24	70	2.9	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R3	33	0.21	68	3.1	<LOQ	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	6.2 ± 1.1	35 ± 9.1	23 ± 14	34 ± 16	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	4.4 ± 1.4	32 ± 2.0	70 ± 64	190 ± 170	n/a	n/a
4	C_w in 5,000 ng/L C_w R1	3.7	0.024	7.6	0.97	<LOQ	<LOQ

Soil #		6:2 FTSA	8:2 FTSA	6:2 FTSaB	8:2 FTSaB	10:2 FTSaB ^a	6:2 FTSaAm
4, cont.	C_w in 5,000 ng/L C_w R2	3.9	0.019	12	0.95	<LOQ	<LOQ
	C_w in 5,000 ng/L C_w R3	4.7	<LOQ	9.5	<LOQ	<LOQ	<LOQ
	C_w in 5,000 ng/L C_w R4	3.9	<LOQ	7.7	<LOQ	<LOQ	<LOQ
	C_w in 60,000 ng/L C_w R1	26	0.18	51	3.1	<LOQ	<LOQ
	C_w in 60,000 ng/L C_w R2	27	0.12	45	<LOQ	<LOQ	<LOQ
	C_w in 60,000 ng/L C_w R3	26	0.14	35	<LOQ	<LOQ	<LOQ
	K_d (5,000 ng/L C_w ; L/kg)	13 ± 1.7	84 ± 35	60 ± 52	61 ± 45	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	12 ± 2.9	120 ± 20	240 ± 270	410 ± 450	n/a	n/a
5	C_w in 5,000 ng/L C_w R1	3.9	0.031	3.6	<LOQ	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R2	4.4	0.044	4.6	<LOQ	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R3	4.1	0.047	5.9	<LOQ	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R4	4.5	0.037	3.9	<LOQ	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R1	33	0.34	36	<LOQ	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R2	33	0.25	31	<LOQ	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R3	43	0.41	32	<LOQ	<LOQ	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	6.4 ± 2.0	30 ± 4.1	74 ± 30	n/a	n/a	n/a
6	C_w in 5,000 ng/L C_w R1	5.1	0.041	2.7	<LOQ	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R2	3.2	0.029	3.0	0.33	<LOQ	<LOQ
	C_w in 5,000 ng/L C_w R3	3.5	0.026	2.2	<LOQ	<LOQ	<LOQ
	C_w in 5,000 ng/L C_w R4	5.8	0.048	4.3	0.97	<LOQ	0.14
	C_w in 60,000 ng/L C_w R1	29	0.25	20	0.85	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R2	28	0.24	26	1.6	<LOQ	0.23
	C_w in 60,000 ng/L C_w R3	25	0.26	26	1.3	<LOQ	0.16
	K_d (5,000 ng/L C_w ; L/kg)	6.6 ± 0.90	28 ± 3.3	90 ± 31	400 ± 150	n/a	840 ± 2.7
1 pH 4	C_w in 5,000 ng/L C_w R1	4.0	0.024	4.9	0.62	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R2	4.9	0.033	6.2	0.80	0.19	<LOD
	C_w in 5,000 ng/L C_w R3	5.3	0.030	7.4	0.44	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R4	5.1	0.028	7.5	0.66	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R1	32	0.22	35	2.2	<LOQ	<LOD

Soil #		6:2 FTSA	8:2 FTSA	6:2 FTSaB	8:2 FTSaB	10:2 FTSaB ^a	6:2 FTSaAm
1 pH 4, cont	C_w in 60,000 ng/L C_w R2	37	0.25	50	3.6	1.1	<LOD
	C_w in 60,000 ng/L C_w R3	34	0.22	45	2.7	0.89	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	6.6 ± 1.8	66 ± 10	79 ± 32	230 ± 23	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	6.3 ± 3.0	53 ± 4.7	64 ± 41	190 ± 62	520 ± 150	n/a
1 pH 7	C_w in 5,000 ng/L C_w R1	4.9	0.047	7.1	0.66	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R2	5.7	0.047	9.2	0.54	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R3	4.2	0.039	6.9	0.41	<LOQ	<LOD
	C_w in 5,000 ng/L C_w R4	5.2	0.043	7.2	0.42	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R1	34	0.38	55	4.2	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R2	34	0.37	50	2.3	<LOQ	<LOD
	C_w in 60,000 ng/L C_w R3	36	0.37	53	2.9	<LOQ	<LOD
	K_d (5,000 ng/L C_w ; L/kg)	5.3 ± 1.6	19 ± 2.7	30 ± 9.7	140 ± 13	n/a	n/a
	K_d (60,000 ng/L C_w ; L/kg)	4.4 ± 2.2	17 ± 1.5	31 ± 16	110 ± 42	n/a	n/a

^aError represents the 95% CI of the n = 4 or n = 3 replicates that incorporates the 95% CI of both K_f and n; The 12:2 FTSaB is not included since all aqueous phase concentrations were below the limit of detection. R1 indicates replicate 1, R2 is replicate 2, etc. LOQ = less than the limit of quantification. <LOD = less than the limit of detection. n/a= Not applicable.

REPORTING AND DISSEMINATION

Reporting

Task 6 for this project included non-experimental subtasks including filing of an interim report and final report. In addition, monthly financial reports and quarterly progress reports were filed as required by SERDP. In progress review presentations were given as scheduled. In addition the findings of this research were disseminated through journal manuscripts, oral presentations, posters and webinars. Three PhD theses also resulted from this work.

Dissemination

The results of this project resulted in 27 public presentations and five webinars as provided below:

1. Will J. Backe and J.A. Field. “Guidelines for applying large-volume injection to environmental samples to overcome the need for solid-phase extraction. “ March, 2011. 241st ACS National Meeting. Anaheim, CA. (Oral Presentation)
2. Will J. Backe and J.A. Field. Large-Volume Injection: “Eliminating chemical redundancy in environmental analysis. “ July 2011. Seminar at the US Environmental Protection Agency Mid-Continent Ecology Division. Duluth, MN. (Oral Presentation)
3. Benjamin Place, Michelle Romero, Jeff Morre, Erika Houtz, David Sedlak, Jennifer Field. “Breaking Down the Legacy: Determination of the Fluorochemical Components of Aqueous Film Forming Foams (AFFF) and Their Potential Environmental Impact.” Platform Presentation. International Conference on Chemistry and the Environment, Zurich, Switzerland, September 2011.
4. Benjamin Place, Erika Houtz, David Sedlak, Liza Alvarez-Cohen, Jennifer Field. “Determination of the Fluorochemical Components of Aqueous Film Forming Foams (AFFF) Used in Military Firefighting.” SERDP Symposium, Washington, D.C., November 29th, 2011.
5. Benjamin Place, Jennifer Field. “Identification of the novel fluorochemical components in U.S. military-use aqueous film-forming foams (AFFFs) using fast atom bombardment and high resolution mass spectrometry.” Platform Presentation. American Chemical Society National Meeting, San Diego, CA, March 26th, 2012.
6. Will J. Backe and J.A. Field. “Analysis of novel and legacy fluorosurfactants in groundwater by inline two-dimensional HPLC MS/MS.” May, 2012, 60th ASMS Conference. Vancouver, BC Canada. (Poster Presentation)
7. Benjamin Place, Michelle Romero, Jeff Morre, Erika Houtz, David Sedlak, Jennifer Field. “Breaking Down the Legacy: Determination of the Fluorochemical Components of Aqueous Film Forming Foams (AFFF) and Their Potential Environmental Impact.” Platform Presentation. International Conference on Chemistry and the Environment, Zurich, Switzerland, September 2011.
8. J. A. Field SETAC North America, Long Beach, CA. Novel and Legacy Fluorosurfactant Contamination in Groundwater from Aqueous Film Forming Foam (AFFF) use at Military Bases. November 11-15, 2012.
9. J. Field. Fluorinated Chemical Research Roadmap Meeting, Sponsored by SERDP,

March 7, 2013 [Invited]

10. J. Field. SERDP/ESTCP Contaminated Groundwater Workshop, Arlington, VA August 13-14, 2013 [Invited]

11. J. Field. SERDP and ESTCP Workshop on Long Term Management of Contaminated Groundwater Sites, October 2013 [Invited]

12. J. Field FCASA Management of Perfluoroalkylated Compounds At Federal Contaminated Sites, Ottawa (Ontario) Canada, February 19-20, 2014 [Invited Speaker].

13. J. A. Field "Per- and Polyfluoroalkyl Substances (PFASs) at US Military Sites" 6th International Workshop Per- and Polyfluoroalkyl Substances – PFASs, Analysis, Fate, Human Exposure, Regulation, June 15-18, 2014, Idstein Germany [Invited Speaker]

14. KA Barzen-Hanson and J Field. "Discovery and Implications of C₂ and C₃ Sulfonates in Groundwater and Aqueous Film-Forming Foams. " Society of Environmental Toxicology and Chemistry (SETAC) North America Annual Meeting. Vancouver, British Columbia. Nov. 9-13, 2014. Poster.

15. J. A. Field "State of the Art Knowledge on Per- and Polyfluoroalkyl Substances (PFASs) DoD Environmental Monitoring and Data Quality Workshop, 2015 Portland, OR April 28 [Invited Speaker]

16. KA Barzen-Hanson, M Kleber, and J Field. "Sorption of Anionic, Zwitterionic, and Cationic Per- and Polyfluoroalkyl Substances to Soil and Sediment. " Fluoros. Golden, CO. July 12-15, 2015. Poster.

17. KA Barzen-Hanson and J Field. "Discovery of Novel Per- and Polyfluoroalkyl Substances in Aqueous Film-Forming Foams and Groundwater. " Invited Seminar at Eawag, Dubendorf, Switzerland. Sept. 18, 2015. Oral.

18. KA Barzen-Hanson, M Kleber, and J Field. "Sorption of Anionic, Zwitterionic, and Cationic Polyfluoroalkyl Substances in AFFF to Soil and Sediment. " International Conference on Chemistry and the Environment 2015. Leipzig, Germany. Sept. 20-24, 2015. Oral.

19. KA Barzen-Hanson, S Roberts, C Higgins and J Field. "Towards Closing the Mass Balance on Per- and Polyfluoroalkyl Substances in Groundwater at Aqueous Film-Forming Foam Impacted Sites. " International Conference on Chemistry and the Environment 2015. Leipzig, Germany. Sept. 20-24, 2015. Poster.

20. KA Barzen-Hanson, S Roberts, C Higgins and J Field. "Discovery of Novel Per- and Polyfluoroalkyl Substances in Aqueous Film-Forming Foams and Groundwater. " Invited Seminar at University of Puget Sound, Tacoma, WA. Nov. 13, 2015. Oral.

21. KA Barzen-Hanson, S Roberts, G Peaslee, C Higgins and J Field. "Closing the Mass Balance on Per- and Polyfluoroalkyl Substances in Groundwater at Aqueous Film-Forming Foam (AFFF) Impacted Sites. " American Chemical Society 251st National Meeting. San Diego, CA. March 13-17, 2016. Oral.

22. KA Barzen-Hanson, M Kleber, and J Field. "Sorption of Anionic, Zwitterionic, and Cationic Polyfluoroalkyl Substances in AFFF to Soil and Sediment. " American Chemical Society 251st National Meeting. San Diego, CA. March 13-17, 2016. Oral.

23. J. A. Field "Per- and Polyfluoroalkyl Substances (PFASs): Frequently Asked Questions and Closing the Mass Balance." Remtek Conference, Warminster, CO, March 1-3 2016 [Invited Keynote Speaker].

24. J. Field and J. Kornuc "Emerging contaminants: Per- and Polyfluoroalkyl Substances (PFASs)" Navy Remediation Innovative Technology Seminar (RITS) Series. Gave

workshops to Navy program managers in Washington DC (Apr 25), Norfolk, VA (Apr 26), San Diego (May 9), Honolulu, HI (May 18), and Jacksonville, FL (June 1) [Invited Speaker]

25. J. A. Field "State of the Art Knowledge on Per- and Polyfluoroalkyl Substances (PFASs) Battelle Chlorinated Conference, Palm Springs, CA. May 22-26, 2016 [Invited Speaker, Session Chair, Organizer]KA Barzen-Hanson, M Kleber, and J Field. "Sorption of Anionic, Zwitterionic, and Cationic Polyfluoroalkyl Substances in AFFF to Soil and Sediment. " Gordon Research Seminar/Conference Environmental Sciences: Water. Holderness, NH. June 25-July 1, 2016. Poster.

26. J. A. Field "State of the Art Knowledge on Per- and Polyfluoroalkyl Substances (PFASs) Australia Air Land Groundwater Association (ALGA) Sponsored Travel: Keynote Speaker, Workshop speaker at EcoForum, Perth Oct 25-27; Keynote speaker at ALGA Events in Melbourne (Oct 31), Sydney (Nov. 1), and Brisbane (Nov. 2) [Invited Speaker]

27. J. A. Field "State of the Art Knowledge on Per- and Polyfluoroalkyl Substances (PFASs) Northwest Environmental Conference and Trade Show. December 6-7, 2016. [Invited Speaker]

Webinars

1. J.A. Field participated in the Environmental Committee of the Society of American Military Engineers (SAME) Webinar on November 7, 2012. The talk was on the topic of Perfluorinated Compounds and had 58 participants from a number of private companies, the military, and federal agencies.
2. Dr. Field and Cornell Long of the Air Force participated in a webinar on October 3, 2013 titled "Perfluorinated Compounds", which was hosted by the Groundwater Resources Association of California (GRACast).
3. Dr. Field gave a talk titled "Analytical Issues & Overview of per- and poly-fluoroalkyl substances (PFAS)" as part of a Society of American Military Engineers (SAME) Webinar titled 'DOD emerging Contaminant Programs: Prioritization, Investigation, and Remediation', held on November 19, 2014. The webinar had 90 participants from a number of private companies, military personnel, and federal agencies.
4. J. Field participated in the SERDP/ESTCP Webinar "Emerging Contaminants: DoD Overview and State of Knowledge on Fluorochemicals and 1,4-Dioxane" on December 3, 2015 as part of SERDP's Tools and Training Webinar Series
5. J. Field participated in the SERDP/ESTCP Webinar "Emerging Contaminants: DoD Overview and State of Knowledge on Per- and Polyfluoroalkyl Substances (PFASs): Analytical and Characterization Frontiers on January 28, 2016 as part of SERDP's Tools and Training Webinar Series
6. J. Field participated in a webinar for Military Legislative Assistants to Senator Murray and Representatives Heck, Smith, DelBene, Kilmer. December 9, 2016.

Peer-reviewed publications (to date)

Place, B., Field, JA. **2012**. Identification of novel fluorochemicals in aqueous film-forming foams (AFFF) used by the US military. Environmental Science and Technology, 46, 7120-7127.

Backe, W.J. and Field, J.A. **2012** Is SPE necessary for environmental analysis? A quantitative

comparison of matrix effects from large-volume injection and solid-phase extraction based methods. 2012. *Environmental Science and Technology*, 46, 6750–6758.

Houtz, E., Higgins, C., Field, J. and Sedlak, D. **2013**. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environmental Science and Technology*, 47, 9342-9349.

Backe, W.J., Christensen, K.E., and Field, J.A. **2013**. Newly-identified cationic, anionic, and zwitterionic fluorinated chemicals in groundwater at US military bases by large volume injection HPLC – MS/MS. *Environmental Science and Technology*, 47, 5226-5234.

McGuire, M.E., Schaefer, C., Richards, T., Backe, W.J., Field, J.A., Houtz, E., Sedlak, D.S., Guelfo, J.L., Wunsch, A., Higgins, C.P. **2014**. Evidence of Remediation-Induced Alteration of Subsurface Poly- and Perfluoroalkyl Substance Distribution at a Former Firefighter Training Area. *Environmental Science and Technology*, 48, 6644-6652.

Harding-Marjanovic, K., Houtz, E., Yi, S., Field, J., Sedlak, D., Alvarez-Cohen, L. **2015**. Aerobic Biotransformation of Fluorotelomer Thioamido Sulfonate (Lodyne™) in AFFF-Amended Microcosms. *Environmental Science and Technology*, 49, 7666–7674.

Barzen-Hanson, K., Field, J.A. **2015**. Discovery and implications of C2 and C3 perfluoroalkyl sulfonates in aqueous film forming foams (AFFF) and groundwater. *Environmental Science and Technology Letters*, 2, 95-99. Selected for Editor's Choice, granting free open access for one year.

Harding-Marjanovic, K.C., Yi, S., Weathers, T.S., Sharp, J.O., Sedlak, D.L., Alvarez-Cohen, L. **2016**. Effects of aqueous film-forming foams (AFFFs) on trichloroethene (TCE) dechlorination by a Dehalococcoides mccartneyi-containing microbial community. *Environmental Science and Technology*, 50, 3352-3361.

Barzen-Hanson, K., Simon, R., Choyke, S., Oetjen, K., McAlees, A., Riddell, N., McCrindle, R., Ferguson, P., Higgins, C., Field, J. **2017**. Discovery of 40 classes of per- and polyfluoroalkyl substances in historical aqueous film-forming foams (AFFF) and AFFF-impacted groundwater. *Environmental Science and Technology*, 51, 2047-2057.

Manuscript in progress

Barzen-Hanson, K.A., Davis, S.E., Kleber, M., Field, J.A. **Submitted**. Sorption of the Fluorotelomer Sulfonates, Fluorotelomer Sulfonamido Betaines, and Fluorotelomer Sulfonamido Amine in National Foam Aqueous Film-Forming Foam to Soil. *Environmental Science and Technology*

Field, J.A. and Seow, J. Review of Fluorotelomer Sulfonates: Properties, Analysis, and Sources of Human and Ecosystem Exposure. **Pending Minor revision**. *Critical Reviews in Environmental Science and Technology*

Awards/Other Impacts

The manuscript by Barzen-Hansen, K. and Field, J.A titled, “Discovery and implications of C2 and C3 perfluoroalkyl sulfonates in aqueous film forming foams (AFFF) and groundwater” that was published in Environmental Science and Technology Letters in 2015 received the Editor’s Choice award and the 2015 Environmental Science and Technology Letters Best Paper Award.

Ms. Barzen-Hanson won a 2015 National Science Foundation Graduate Research Fellowship Program Fellowship for her proposal titled “Polyfluorinated Alkyl Substances at Aqueous Film-Forming Foam (AFFF) Impacted Sites: Identification of Unknowns and Effective Chemical Oxidation Remediation Development”.

The C3-perfluoropropane sulfonate reported in the 2015 Environmental Science and Technology Letters paper was synthesized for commercial sale by Wellington Laboratories in 2016.

Summary and Conclusions

Key points from Task 1:

- Over 50 classes of PFASs, comprised of several individual homologs, were identified in AFFF formulations and groundwater over the course of this project
- Perfluorinated chain lengths ranged from 4 to 12 but the major perfluorinated chain length had six perfluorinated carbons.
- Carboxylates (e.g., PFOA) and FTSA were only minor components of ECF and telomer-based AFFF formulations, respectively.
- Many of the newly-identified classes of PFASs are cationic or zwitterionic and this may impact their association with sediments and soils in the field, as well as their toxicity.
- TOP assay data indicate that AFFF formulations generate only carboxylates upon oxidation.
- Determining the agreement between the TOP assay and the estimated concentration of newly-identified PFASs has yet to be determined (e.g., mass balance on the TOP assay).

Key Points from Task 2:

- After development of analytical methodology for newly-identified PFASs, PFCAs, and PFASs, and FTASs remain the most abundant PFASs in groundwater.
- Up to half of precursors in groundwater, sediment, and soil, as measured by the TOP assay, cannot be accounted for by precursors measured directly by LC-MS/MS.
- Application of the TOP assay to field samples indicates that PFASs in groundwater are likely characterized by chain lengths < C8 while precursors in soil/sediments are characterized by longer chain lengths

Key Points from Task 3

- 6:2 and 8:2 FTSA degradation to PFCAs occurred in aerobic microcosms prepared with pristine soils and National Foam AFFF.
- 6:2 FTTAoS degradation to FTSA and PFCAs occurred in aerobic enrichments derived from

pristine soil previously cultured with National Foam AFFF.

- Rapid degradation of 6:2 FTAAoS to FTSA and PFCAS occurred in aerobic enrichments prepared with contaminated soil from Ellsworth Air force Base.
- 6:2 FTAAoS degradation was observed in anaerobic microcosms under nitrate-, sulfate-, iron-reducing, and methanogenic conditions with the greatest degradation rates were observed under nitrate-reducing conditions.
- The formation of perfluoroalkyl thiocarboxylate (FTTCA) was identified for the first time as a transformation product of FTAAoS.
- 3M AFFF and DGBE are capable of promoting reductive dechlorination of TCE in enriched anaerobic microbial communities when they are provided as the sole carbon and energy source.
- Significant quantities of hydrogen are produced when National Foam AFFF is amended to the cultures; however, reductive dechlorination does not occur after 2 weeks with this specific AFFF formulation.
- The microbial community of *D. mccartyi* demonstrated the capacity to ferment the non-fluorinated organic components (e.g., DGBE) in all four AFFF formulations tested to hydrogen and acetate.
- The products formed varied in concentration with the type of AFFF formulation tested. In the presence of 3M AFFF, TCE was dechlorinated but not in the presence of Ansul and National Foam AFFFs.
- This research indicate that the DGBE stimulates TCE dechlorination while PFAS in some AFFF formulations inhibited reductive dechlorination.

Key Points from Task 4

- Low K_d values for the anionic 6:2 FTSA suggest that the 6:2 FTSA is highly mobile in groundwater.
- Higher K_d values indicate that the anionic 8:2 FTSA, zwitterionic FTSAbs, and cationic 6:2 FTSAam are more likely to be associated with soil and sediment of source zones.
- Complete removal of the cationic FTSAam indicates potential for strong sorption to source zone soils and sediments at some sites.
- In contrast, measurement of cationic PFASs in groundwater indicates the potential to impact surface water sources and cationic surfactants are more toxic to aquatic species.
- Removal of zwitterionic and cationic PFASs from drinking water sources by conventional technology (GAC) is unknown.
- The lack of correlations between the sorption of anionic FTSA, zwitterionic FTSAbs, and cationic 6:2 FTSAam and parameters including organic carbon content, CEC, and AEC, indicates that the bulk parameters do not adequately predict sorption. More research is needed the factors that control their sorption.
- Sorption of additional PFASs in AFFF to a soil already saturated with PFASs may increase the sorption of the added PFASs (e.g., multi-layer sorption).
- Conditions (e.g. pH and ionic strength) that promote desorption of zwitterionic and cationic PFASs need to be determined to determine potential for source zone sediments to act as long-term PFAS sources.

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